ISOLATED DIMERIC FIBROBLAST ACTIVATION PROTEIN ALPHA, AND USES THEREOF

RELATED APPLICATION

This application is a continuation-in-part of Serial No. 08/230,491, filed April 20, 1994, now pending and incorporated by reference.

FIELD OF THE INVENTION

This invention relates to certain molecules associated with cancer tissues and reactive tumor stromal cells. More particularly, it relates to fibroblast activation protein alpha ("FAPα" hereafter) molecules. A monomeric form of the molecule has previously been identified immunochemically, but nucleic acid molecules coding for it had not been isolated or cloned nor have dimers been identified. These, inter alia, are features of the invention. The monomeric protein has a molecular weight of from about 88 to about 95 kilodaltons as determined by SDS-PAGE of boiled samples. The dimer has a molecular weight of about 170 kilodaltons as determined by SDS-PAGE of unboiled samples. FAPα is characterized by a number of features and properties which are shared by and characteristic of membrane bound enzymes, suggesting very strongly that it, too, is a membrane bound enzyme. The nucleic acid molecules, which are a key part of the invention, are useful both as probes for cells expressing FAPα, and as starting materials for recombinant production of the protein. The FAPα protein can then be used to produce monoclonal antibodies specific for the protein and are thus useful diagnostic agents themselves. They also have additional uses, including uses related to enzymatic functions, as described herein.

BACKGROUND AND PRIOR ART

The invasive growth of epithelial cancers is associated with characteristic cellular and molecular changes in the supporting stroma. For example, epithelial cancers induce the formation of tumor blood vessels, the recruitment of reactive tumor stromal fibroblasts, lymphoid and phagocytic

infiltrates, the release of peptide mediators and proteolytic enzymes, and the production of an altered extracellular matrix (ECM). See, e.g., Folkman, Adv. Cancer Res. 43: 175-203 (1985); Basset et al., Nature 348: 699-704 (1990); Denekamp et al., Cancer Metastasis Rev. 9: 267-282 (1990); Cullen et al., Cancer Res. 51: 4978-4985 (1991); Dvorak et al., Cancer Cells 3: 77-85 (1991); Liotta et al., Cancer Res. 51: 5054s-5059s (1991); Garin-Chesa et al., J. Histochem. Cytochem. 37: 1767-1776 (1989). A highly consistent molecular trait of the stroma in several common histologic types of epithelial cancers is induction of the fibroblast activation protein (FAPα), a cell surface glycoprotein with an observed M_r of 95,000 originally discovered with a monoclonal antibody, mAb F19, raised against proliferating cultured fibroblasts. See Rettig et al., Cancer Res. 46: 6406-6412 (1986); Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. USA 87: 7235-7239 (1990); Rettig et al., Cancer Res. 53: 3327-3335 (1993). Each of these four papers is incorporated by reference in its entirety.

Immunohistochemical studies such as those cited supra have shown that FAPα is transiently expressed in certain normal fetal mesenchymal tissues but that normal adult tissues are generally FAPα$^-$. Similarly, malignant epithelial, neural and hematopoietic cells are generally FAPα$^-$. However, most of the common types of epithelial cancers, including >90% of breast, lung, skin, pancreas, and colorectal carcinomas, contain abundant FAPα$^+$ reactive stromal fibroblasts. Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7239 (1990). The FAPα$^+$ tumor stromal fibroblasts almost invariably accompany tumor blood vessels, forming a distinct cellular compartment interposed between the tumor capillary endothelium and the basal aspect of malignant epithelial cell clusters. While FAPα$^+$ stromal fibroblasts are found in both primary and metastatic carcinomas, benign and premalignant epithelial lesions, such as fibroadenomas of the breast and colorectal adenomas only rarely contain FAPα$^+$ stromal cells. In contrast

to the stroma-specific localization of FAPα in epithelial neoplasms, FAPα is expressed in the malignant cells of a large proportion of bone and soft tissue sarcomas. (Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988)). Finally, FAPα$^+$ fibroblasts have been detected in the granulation tissue of healing wounds (Garin-Chesa et al., supra). Based on the restricted distribution pattern of FAPα in normal tissues and its uniform expression in the supporting stroma of many epithelial cancers, clinical trials with ^{131}I-labeled mAb F19 have been initiated in patients with metastatic colon cancer (Welt et al., Proc. Am. Assoc. Cancer Res. 33: 319 (1992); Welt et al. J. Clin. Oncol. 12: 1561-1571 (1994)) to explore the concept of "tumor stromal targeting" for immunodetection and immunotherapy of epithelial cancers.

Rettig et al., Int. J. Cancer 58: 385-392 (1994), incorporated by reference, discusses the FAPα molecule and its features. Rettig et al postulate that FAPα is found in high molecular weight complexes in excess of 400 kilodaltons, but do not discuss the possibility of dimeric molecules, nor does the paper elaborate on the specific enzymatic properties of the molecule.

The induction of FAPα$^+$ fibroblasts at times and sites of tissue remodeling during fetal development, tissue repair, and carcinogenesis is consistent with a fundamental role for this molecule in normal fibroblast physiology. Thus, it is of interest and value to isolate and to clone nucleic acid molecules which code for this molecule. This is one aspect of the invention, which is described in detail together with other features of the invention, in the disclosure which follows. Further aspects of the invention include the dimeric FAPα molecules, and the exploitation of the properties of these molecules. These features are also elaborated upon hereafter.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 compares the deduced amino acid sequence for FAPα, and the known sequence of CD26. The alignment has been optimized.

Figures 2A-2H, inclusive, display immunohistochemical detection of FAPα and CD26 in various tissues. In figures 2A and 2B, breast cancer is studied, for FAPα (figure 2A), and CD26 (figure 2B). In figures 2C and 2D, malignant fibrous histiocytoma is studied, for FAPα (figure 2C), and CD26 (figure 2D). Dermal scar tissue is examined in figures 2E (FAPα), and 2F (CD26). Renal cell carcinoma is studied in figure 2G (FAPα), and 2H (CD26).

Figure 3 presents some of the data generated in experiments which showed that FAPα had extracellular matrix (ECM) protein degrading activity. When zymographic detection of gelatin degrading extracts of 293-FAP was carried out, the active substance was found to have a molecular weight of about 170 kD, via SDS-PAGE, using unboiled samples to preserve enzyme activity.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

Fibroblast cell line WI-38 had been observed, previously, to react with mAb F19 (Rettig et al., Canc. Res. 46: 6406-6412 (1986); Rettig et al., Proc. Natl. Acad. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7239 (1990); Rettig et al., Canc. Res. 53: 3327-3335 (1993)). It was used in the experiments which follow.

A cDNA library was prepared from WI-38, using well known techniques and commercially available materials. Specifically, the library was constructed in expression vector pCDNAI, using the Fast Track mRNA isolation kit, and Librarian cDNA phagemid system. Once the library was prepared, the vectors were electroporated into cell line E. coli MC 1061/P3. The pCDNAI expression vector contains an antibiotic resistance gene, so the E. coli were selected via antibiotic resistance. The colonies which were resistant were then used in further experiments. The plasmid DNA from the colonies was obtained via alkaline lysis and purification on $CsCl_2$, in accordance with Sambrook et al, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab, Cold Spring Harbor, N.Y. 2d Ed. 1989). The technique is well known to the art, but is

incorporated by reference herein.

Once the plasmid DNA was isolated, it was used to transfect COS-1 cells, which were then cultured for forty-eight hours, after which these were tested with antibody coated dishes. The mAbs used included F19, as described by Rettig et al., (1986), supra, which is incorporated by reference in its entirety. As COS-1 cells are normally FAPα⁻, any positive results indicated the presence of the coding sequence. The immunoselection protocol was that of Aruffo et al., Proc. Natl. Acad. Sci USA 84: 3365-3369 (1987), incorporated by reference herein.

Plasmid DNA from positive clones was recovered, in accordance with Hirt, J. Mol. Biol. 26: 365-369 (1967), reintroduced into E. coli MC 1061/P3, and reselected in COS-1 cells.

The protocol presented herein was followed for four rounds. After this, the plasmid DNA of 50 isolated bacterial colonies was purified, using the Qiagen plasmid kit. Of the colonies, 27 clones were found to contain identical 2.8 kb inserts, as determined by EcoRI restriction enzyme mapping. Several of these were found to contain FAPα-specific cDNA as determined by transient expression in COS-1 cells and direct immunofluorescence staining with mAb F19. One of these clones, i.e., "pFAP.38" was selected for further study, as elaborated upon infra.

Example 2

Once pFAP.38 had been identified, it was tested together with a vector coding for known cell surface marker CD26 ("pCD26"), as well as with control vector pCDNA I.

In these experiments, COS-1 cells were transfected with one of pFAP.38, pCD26, or pCDNAI. After forty-eight hours, the transfectants were tested, using the well known MHA rosetting assay for cell surface antigen expression. In these experiments, mAb F19, which is FAPα specific, was used, together with mAb EF-1, which is CD26 specific. Also used were four other FAPα specific mAbs, i.e., FB23, FB52, FB58 and C48. Also tested were two cancer cell lines, which are known

to react with mAb F19 (SW872 liposarcoma), or EF-1 (SK-OV6 ovarian cancer). The results are set forth in Table 1, which follows.

Table 1. Cell surface expression of multiple FAPα epitopes and CD26 in human cells and COS-1 cell transfectants

	Cell surface antigen expression					
Target cell	F19	FB23	FB52	FB58	C48	EF-1
Human cells						
SW872 liposarcoma	>95%	>95%	>95%	>95%	>95%	-
SK-OV6 ovarian cancer	-	-	-	-	-	>95%
COS-1 transfectants						
COS·pCDNAI control	-	-	-	-	-	-
COS·pFA P 38	40%	30%	40%	20%	20%	-
COS·pCD26	-	-	-	-	-	40%

Example 3

Immunoprecipitation studies were then carried out to identify the antigen being targeted by the antibodies.

Cells were metabolically labelled with Trans ^{35}S-label, (ICN), extracted with lysis buffer (0.01 M Tris-HCl/0.15 M NaCl/0.01 M $MgCl_2$/0.5% Nonidet P-40/aprotinin (20 ug/ml)/2 mM phenylmethyl- sulfonyl fluoride), and then immunoprecipitated. The protocols used are all well known, as will be seen by reference to Rettig et al., Canc. Res. 53: 3327-3335 (1993); and Fellinger et al., Canc. Res. 51: 336-340 (1991), the disclosures of which are all incorporated by reference in

their entirety. Precipitating mAbs were negative control mouse Ig, mAb F19, or EF-1. Control tests were carried out with mock transfected COS-1 cells. Following immunoprecipitation, the immunoprecipitates were boiled in extraction buffer and separated by $NaDOdSO_4$/PAGE, under reducing conditions. In some experiments, an additional test was carried out to determine whether or not the immunoprecipitated material was glycosylated. In these experiments, cell extracts were fractionated with Con A-SEPHAROSE prior to immunoprecipitation. Following immunoprecipitation, but prior to fractionation on $NaDodSO_4$/PAGE, these precipitates were digested with N-Glycanase.

The results showed that, in COS-1 cells, pFAP.38 directs expression of an 88 kd protein species (as determined via SDS-PAGE), which is slightly smaller than the 95 kd FAPα species produced by SW872, or cultured fibroblasts. Digestion with N-Glycanase produced peptides of comparable size (i.e., 74 kd versus 75 kd), showing that the glycosylation of the FAPα protein in COS-1 cells is different than in the human cell lines.

Example 4

Classic Northern blot analysis was then carried out, using the mRNA from FAPα$^+$ fibroblast cell lines WI-38 and GM 05389, and FAPα$^-$ovarian cancer cell line SK-OV6. Using the procedures of Sambrook et al., supra, five micrograms of mRNA from each cell line were tested. The probes used were ^{32}P labelled, and were prepared from a 2.3 kb ECO I fragment of pFAP.38, a 2.4 kb Hind III fragment of CD26, and a 1.8 kb BamHI fragment of γ-actin cDNA. These fragments had been purified from 1% agarose gels.

The extracts of FAPα$^+$ fibroblast strains showed a 2.8 kb FAP mRNA species, but extracts of SK-OV6 do not. A γ-actin mRNA species (1.8 kb), was observed in all species.

Example 5

The cDNA identified as coding for FAPα was subjected to more detailed analysis, starting with sequencing. The classic

Sanger methodology, as set forth in Proc. Natl. Acad. Sci. USA 74: 5463-5467 (1977), was used to sequence both strands of the cDNA. Once this was secured, an amino acid sequence was deduced therefrom. This information is presented in SEQ ID NO: 1. The sequence was then compared to the known amino acid sequence of CD26 (Morimoto et al., J. Immunol. 143: 3430-3437 (1989)). Figure 1 presents the comparison, using optimized sequence alignment. Any gaps in the comparison are indicated by asterisks, while identical amino acids are shown by dashes in the CD26 sequence. A hydrophobic, putative transmembrane sequence is double underlined, while potential N-glycosylation sites are single underlined.

The sequence analysis shows a 2812 base pair insert, wherein 2277 base pairs constitute the open reading frame. This ORF extends from start codon ATG at nucleotide 209, to stop codon TAA at 2486.

The deduced polypeptide is 760 amino acids long, and has a molecular weight of 87,832. In contrast, N-Glycanase digested, immunopurified FAPα was reported to have an estimated M_r of 75,000 on $NaDodSO_4$/PAGE (Rettig et al., Canc. Res. 53: 3327-3335 (1993)). A GenBank data base search was carried out. The most closely related genes found were those encoding dipeptidyl peptidase IV homologues (DPPIV; EC 3.4.14.5), with human DPPIV (also known as T-cell activation antigen CD26), showing 61% nucleotide sequence identity, and 48% amino acid sequence identity.

The second set of related genes are human, rat, and bovine homologues of DPPX, a gene of unknown function widely expressed in brain and other normal tissues. The predicted human DPPX gene product shows about 30% amino acid sequence identity with FAPα and CD26. The FAPα molecule exhibits structural features typical of type II integral membrane proteins, including a large COOH-terminal extracellular domain, a hydrophobic transmembrane segment, and a short cytoplasmic tail. The putative extracellular domain contains five potential N-glycosylation sites, eleven cysteine residues (eight of which are conserved between FAPα and CD26), and

three segments corresponding to highly conserved catalytic domains characteristic of serine proteases, such as DPPIV. These conserved sequences are presented in Table 2, which follows. Comparisons to DPPIV and DPPX were made via Morimoto et al., supra; Wada et al., Proc. Natl. Acad. Sci. USA 89: 197-201 (1992); Yokotani et al., Human Mol. Genet. 2: 1037-1039 (1993).

Example 6

An additional set of experiments were carried out to determine whether FAPα related sequences are present in non-human species. To do so, human, mouse, and Chinese hamster genomic DNA was digested using restriction enzymes, and tested, via Southern blotting, using the 2.3 kb fragment, labelled with ^{32}P, describes supra. Hybridization was carried out using stringent washing conditions (0.1 x SSC, 0.1% NaDodSO$_4$, 68°C). Cross-hybridization was readily observed with both the mouse and hamster DNA, suggesting the existence of highly conserved FAPα homologues. In control experiments using the CD26 cDNA fragment described supra, no evidence of cross hybridization was observed.

Example 7

The CD26 molecule shares a number of biochemical and serological properties with FAPβ, which is a previously described, FAPα associated molecule having a molecular weight of 105 kd, and is found on cultured fibroblasts and melanocytes (Rettig et al., Canc. Res. 53: 3327-3335 (1993)). Cotransfection experiments were carried out to determine whether FAPβ is a CD26 gene product. To test this, the same protocols were used which were used for transfection with pFAP.38 or pCD26, as described supra, but using the two vectors. The results presented supra showed that cotransfection efficiency was about 40% for each vector, so about 10-20% of cell should be cotransfected.

Following cotransfection, the COS-1 cells were Trans ^{35}S-labeled, as described supra, then lysed, also as described supra.

The resulting cell extracts were separated on Con A

SEPHAROSE, and the antigen (FAPα and/or CD26) were recovered in the Con A-bound fraction. The bound fraction was eluted with 0.25 M α-D-mannopyranoside. Immunoprecipitation was then carried out, as described supra, and the precipitates were separated on $NaDodSO_4$/PAGE, also as discussed supra.

Those cells transfected only with pFAP.38 produced FAPα, but not FAPβ (determined from mAb F19 immunoprecipitates). They also produce no CD26 antigen (tested with EF-1). Those cells transfected with pCD26 alone produce CD26 but no FAPα. Cotransfectants produce CD26 and FAPα/FAPβ heteromers, as determined in the mAb F19 precipitates. This result provides direct evidence that FAPβ is a CD26 gene product.

Example 8

It has been observed previously that some cultured human cell types coexpress FAPα and CD26, and show FAPα/CD26 heteromer formation. In vivo distribution patterns of FAPα and CD26, however, as determined in previous immunohistochemical studies, appeared to be non-overlapping. (See Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7329 (1990); Rettig et al., Canc. Res. 53: 3327-3335 (1993); Stein et al., in Knapp et al., eds. Leukocyte typing IV-white cell differentiation antigens, pp 412-415 (Oxford University Press, N.Y. 1989), pp. 412-415; Möbious et al., J. Exp. Immunol. 74: 431-437 (1988)). In view of the potential significance of FAPα/CD26 coassociation, tissue distribution was reexamined, via side by side immunohistochemical staining of normal tissues and lesional tissues known to contain FAPα$^+$ fibroblasts or FAPα$^+$ malignant cells.

To test the samples, they were embedded in OCT compound, frozen in isopentane precooled in liquid nitrogen, and stored at -70°C until used. Five micrometer thick sections were cut, mounted on poly-L-lysine coated slides, air dried, and fixed in cold acetone (4°C, for 10 minutes). The sections were then tested with mAbs (10-20 ug/ml), using the well known avidin-biotin immmuno-peroxidase method, as described by, e.g., Garin-Chesa et al., J. Histochem. Cytochem. 37: 1767-

1776 (1989); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7239 (1990); Rettig et al., Canc. Res. 53: 3327-3335 (1993); Garin-Chesa et al., Am. J. Pathol. 142: 557-567.

The results are shown in figure 2. Breast, colorectal, pancreas and lung carcinomas showed strong expression of FAPα and no CD26 was found (see figures 2A and 2B). Five FAPα$^+$ sarcomas, including malignant fibrous histiocytoma (figures 2C and 2D), were tested, and there was no expression of CD26. Examination of reactive fibroblasts of healing dermal wounds (figures 2E, 2F), showed abundant expression of both FAPα and CD26. The three renal carcinomas tested (figures 2G, 2H), showed expression of CD26 in malignant epithelium. FAPα was absent from malignant epithelial cells, and showed low expression in the stroma of these carcinomas.

Example 9

A mammalian cell line, transfected with a FAPα encoding cDNA, was prepared.

Human embryonic kidney cell line 293 is well known and widely available from, e.g., the American Type Culture Collection.

Samples of 293 were maintained, in an incubator, at 37°C, in an atmosphere of 95% air, and 5% CO_2. The cells were cultured in a 50:50 mixture of Dulbecco's modified minimal essential medium and Ham's F12 medium, augmented with 10% fetal bovine serum, penicillin and streptomycin. Following the procedures described by Ustar et al., Eur. Mol. Biol. J. 1991, and/or Park et al., J. Biol. Chem. 169: 25646-25654 (1994), both of which are incorporated by reference, cDNA for FAPα (i.e., SEQ ID NO: 1), was transfected into the 293 cells. Details of the cDNA vector are provided, supra (pFAP.38). Transfectants were selected for resistance to antibiotics (200 ug/ml Geneticin), and were then maintained in selection medium, containing Geneticin.

Individual colonies of resistant cells were picked, grown to confluence in 6 well tissue culture plates, and were tested for FAPα expression in an immunofluorescence assay (IFA), using FAPα specific monoclonal antibody F19 as described

supra.

Those colonies which expressed FAPα were expanded, and monitored by indirect IFA and cytofluorometric analysis, also as set forth, supra.

The IFAs were positive for the transfectants, referred to hereafter as cell line 293-FAP, but were negative for parental line 293.

Example 10

In order to confirm that recombinant FAPα was, in fact, being produced, a series of immunoprecipitation experiments were carried out. These followed the methods of Park, et al., supra, and Rettig et al., Canc. Res. 53: 3327-3335 (1993), both of which are incorporated by reference. Essentially, ^{35}[S] methionine labelled cell extracts were combined with monoclonal antibody F19, in the manner described supra. Precipitates were then boiled in extraction buffer and run on SDS-PAGE gels, using, as a negative control, mouse IgG1. Both cell line 293-FAP, and non transfected line 293 were tested. The results indicated clearly, that recombinant FAPα was produced by the transfected cell line 293-FAP. This was determined by immunoprecipitation analyses, using FAPα specific monoclonal antibody F19.

Example 11

The ability to produce recombinant FAPα permitted further study of the molecule's properties. Specifically, given the structural features outlined in the prior examples, experiments were designed to determine if FAPα possesses enzymatic activities. The experiments were designed to test whether or not FAPα had extracellular matrix (ECM) protein degrading activity.

Extracts of 293-FAP cells were prepared, using an extraction buffer (0.15M NaCl, 0.05M Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 percent Triton X-114), were cleared by centrifugation (4,000xg, 10 minutes at 4°C), and phase partitioned at 37°C for 10-20 minutes. This was followed by further centrifugation (4000xg, 20 minutes at 20-25°C). Detergent phases were diluted with buffer (0.15 M NaCl, 0.05 M Tris-HCl

pH 7.4, 5 mM $CaCl_2$, 5 mM $MgCl_2$, 0.75% Empigen BB), and separated on concanavalin A-Sepharose following Rettig et al., supra. Any concanavalin A bound fractions were eluted with 0.25M methyl-α-D-mannopyranoside in elution buffer 0.15 M NaCl,0.05 M Tris-HCl, pH 7.4, 5mM $CaCl_2$, 5 mM $MgCl_2$, 0.1% Triton X-100), mixed with zymography sample buffer (0.25 M Tris-HCl, pH 6.8, 8% SDS, 40% glycerol, 0.01% bromophenol blue), at a 3:1 ratio, and used for further analysis.

Aliquots of sample were loaded onto polyacrylamide gels containing 0.1% of either of gelatin or casein. Electrophoresis was then carried out in a Biorad Mini-Protein II system, at 20 mA constant current for 1.5 - 2 hours, until the bromophenol blue due fronts of samples had reached the lower end of the gel. The gel was removed and incubated for one hour at 20-25°C in a 2.5% aqueous solution of Triton X-100 on a rotary shaker. The Triton X-100 solution was decanted, and replaced with enzyme buffer (0.05M Tris-HCl, pH 7.5, 0.2M NaCl, 5 mM $CaCl_2$, 5 mM $MgCl_2$, 0.02% Brij 35). The gel was then incubated at 37°C or 41°C, followed by staining or destaining at room temperature. Gels were stained with 0.5% of Coomassie Brilliant Blue G-250 in an aqueous solution of 30% methanol and 10% acetic acid for 15, 30, and 60 minutes, respectively. Subsequently, gels were incubated for 15 minutes in an aqueous solution of 30% CH_3OH and 5% glycerol, followed by drying between sheets of cellophane.

Gelatinase activity was evaluated in accordance with Kleiner et al., Anal. Biochem. 218: 325-329 (1994), incorporated by reference in its entirety. This is a routine assay used to determine whether or not a protease capable of digesting gelatin is present. Labelled molecular weight standard were run on the same gels, under reducing conditions, for molecular weight determinations.

Proteolytic activity for defined amino acid sequence motifs were tested, using a well known membrane overlay assay. See Smith et al, Histochem. J. 24(9): 637-647 (1992), incorporated by reference. Substrates were Ala-Pro-7-amino-4-trifluoromethyl coumarin, Gly-Pro-7-amino-4-trifluoromethyl

coumarin, and Lys-Pro-7-amino-4-trifluromethyl coumarin.

The results of these experiments are depicted, in part, in figure 3. This figure shows zymographic detection of gelatin degrading activity, in the cell extracts. See Kleiner et al., supra. A protein species of approximately 170 kilodaltons, as determined by SDS-PAGE, was observed to have gelatin degrading activity. This species, which was found in the 293-FAP cell line, but not in untransfected 293 cells, is thus identified as FAPα. The molecular weight is consistent with a dimer, i.e., a dimeric FAPα molecule.

The proteolytic activity described herein where gelatin is the substrate, was not observed when casein was the substrate.

Example 12

Further studies were then undertaken in order to characterize the 170 kD FAPα dimer further. Specifically, the experiments described in example 11 were repeated, except that 5% of 2-mercaptoethanol or 5 um iodoacetamide was added to the extracts prior to SDS-PAGE, or ethylenediamine N,N,N',N'-tetraacetic acid (10 mM) was added to the incubation buffer used for gelatin zymography. None of these treatments abolished the enzymatic activity. In contrast, heating at 100°C for five minutes prior to SDS-polyacrylamide gel electrophoresis abolished the gelatin-degrading activity.

Further work, using a membrane overlay assay, described by, e.g., Smith et al., Histochem J. 24(9): 643-647 (1992), incorporated by reference, revealed that the FAPα dimers were able to cleave all of the Ala-Pro, Gly-Pro, and Lys-Pro dipeptides tested.

In further experiments, a fusion protein was produced which comprised the extracellular domains of both FAPα and murine CD8 proteins. This chimeric protein was produced in a baculovirus system in insect cells. The chimeric protein exhibited the same enzymatic activity as FAPα, using the model discussed supra.

Example 13

Two quantitative assays for FAPα enzyme activity were developed using Ala-Pro-7-amino-4-trifluoromethyl coumarin (Ala-Pro-AFC) as the substrate. In the first assay format, membrane extracts of FAPα-expressing cells were mixed with a 5-10 fold volume of reaction buffer (100mM NaCl, 100mM Tris pH 7.8), and added to an equal volume of 0.5mM Ala-Pro-AFC in reaction buffer followed by an incubation for one hour at 37°C. Release of free AFC was then measured in a fluorimeter using a 395nm excitation / 530nm emission filter set. The membrane extracts analyzed in this assay format were derived from either 293-FAPα cells (293 cells stably transfected with vector FAP.38 described supra) or HT1080-FAPα cells (HT1080 cells stably transfected with vector FAP.38). Negative control experiments assessing FAPα-specific activities were carried out with membrane extracts prepared from the respective parental 293 or HT1080 cell lines. In the second assay, FAPα was isolated from 293-FAPα or HT1080-FAPα membrane extracts via an antibody specific for FAPα. Ninety-six well ELISA plates were coated overnight at 4°C with 1ug/ml F19 monoclonal antibody in phosphate-buffered saline (PBS). In the case of CD8-FAPα discussed infra plates were coated with F19 antibody as above or with 1ug/ml rat anti-mouse CD8 overnight at 4°C. Wells were then washed with wash buffer (PBS, 0.1% Tween 20). Excess binding sites were blocked with blocking buffer (5% bovine serum albumin in PBS) for 1 hour at room temperature. Blocking buffer was removed; membrane extracts of 293-FAPα expressing cells or control cells were added and incubated for 1 hour at room temperature. The unbound material was removed, wells were washed with wash buffer, and FAPα activity was assayed using 100 ul Ala-Pro-AFC (0.5 mM Ala-Pro-AFC in reaction buffer) for one hour at 37°C. Release of free AFC was measured as above. Binding of mab F19 to FAPα did not measurably affect its enzymatic activity.

Example 14

Using assays for FAPα enzyme activity, described supra an inhibitor of FAPα enzymatic activity has been identified. This inhibitor is (S)-Valylpyrrolidine-2(R)-boronic acid (Snow

et al., J. Am. Chem Soc.(1994) 116:10860-10869), referred to here as ValboroPro. ValboroPro inhibits cleavage of Ala-Pro-AFC by FAPα with an IC_{50} of 0.11 uM. ValboroPro also inhibits the gelatinolytic activity of FAPα at a concentration of 100 uM. The specificity of ValboroPro for FAPα was demonstrated in tests with an unrelated serine protease, trypsin. No inhibition of bovine trypsin by ValboroPro (up to 100 uM) was observed when assayed with carbobenzoxy-L-valinyl-glycinyl-L-arginyl-4-nitranilide acetate as substrate.

Example 15

The identification of specific, structural requirements for the enzymatic activities of FAPα facilitates the development of molecules which can bind to and/or inhibit FAPα. To examine whether the serine residue at position 624 of the predicted amino acid sequence of FAPα polypeptide is critical for its enzymatic function, site-directed mutagenesis according to Zoller, et al DNA 3:479-488 (1984) was performed using standard polymerase chain reaction methods. The TCC codon coding for serine 624 in the FAPα cDNA was replaced with GCG, resulting in alanine at this position. The altered DNA was reintroduced into the FAP.38 vector and transfected into 293 cells as described supra. Geneticin-resistant colonies were selected and examined by indirect IFA for FAPα expression using mAb F19 as well as other FAPα specific antibodies described by Rettig, et al., J. Cancer 58:385-392 (1994) as set forth, supra. No differences in binding of the anti-FAPα antibodies to the mutant FAPα expressing cells were observed as compared to wild type FAPα transfected cells. The presence of the mutation was confirmed through amplification of genomic DNA and restriction enzyme digestion performed with several clones of transfected cells. To assess the enzymatic activity of mutant FAPα, the following tests were performed. Membrane extracts were prepared from three independent positive clones and equal amounts of FAPα protein (as determined in a double-determinate ELISA assay using two anti-FAPα antibodies that recognize distinct FAPα epitopes) were examined in the gelatinolytic and Ala-Pro-AFC capture assays.

Both the gelatinolytic activity and the activity in the capture assay of isolated mutant FAPα were reduced to undetectable levels compared to wild type FAPα, confirming the role of the canonical serine in the catalytic triad for both observed enzymatic activities.

Example 16

A fusion protein was generated to obtain secreted, water-soluble FAPα enzyme. In this fusion protein, the extracellular domain of CD8, consisting of the first 189 amino acids of murine CD8, was linked to the extracellular domain of FAPα (amino acids 27 to 760), as described by Lane et al., J. Exp. Med. 177:1209 (1993)using standard polymerase chain reaction protocols and inserted in commercially available pVL1393 vector. Transfection of Sf9 cells with this vector and amplification of the resulting recombinant baculovirus were performed as described (Baculovirus Expression Vectors, O'Reilly, Miller, and Luckow, Oxford University Press, 1994). The CD8-FAP fusion protein was isolated in a two step purification from the spent medium of High Five™ cells infected with CD8-FAPα baculovirus for four days. Cells and virus were removed by ultracentrifugation, the supernatant was passed through a column containing Heparin-Sepharose (Pharmacia) and eluted stepwise with 0.6, 1.0, and 2.0 M NaCl in 10mM phosphate, pH 7. Active fractions from the 1.0 and 2.0 M eluates were pooled and concentrated using an YM-10 filter and 26/60 Superdex-200 gel filtration column. Activity was observed in a high molecular weight peak which, when subjected to N-terminal gas phase sequencing, was confirmed to be CD8-FAPα. In gelatinolytic assays, activity greater than 200kD in the gelatinolytic assay was detected when purifieed CD8-FAPα was tested, consistent with the higher predicted molecular weight of the fusion protein.

Example 17

The presence of structural and functional homologues in non-human species has been ascertained. For example, the cDNA for mouse FAPα has been cloned and characterized. Examination of the predicted amino acid sequence of the homologous mouse

FAPα cDNA sequence (EMBL accession number Y10007) reveals a high degree of conservation of FAPα across species. The two proteins are 89% identical and the catalytic triad is conserved between human FAPα and mouse FAPα. The high degree of conservation and simlilar tissue expresion suggests that FAPα from nonhuman sources may be functionally equivalent to human FAPα. This conclusion is confirmed by the finding that a CD8-murine FAPα fusion protein similar in design to CD8-human FAPα also demonstrates the expected dipeptidylpeptidase enzymatic activity using Ala-Pro-AFC as substrate.

The foregoing examples describe an isolated nucleic acid molecule which codes for fibroblast activating protein alpha ("FAPα"), as well as dimeric forms of the molecule, and uses thereof. The expression product of the sequence in COS-1 is a protein which, on SDS-PAGE of boiled samples, shows a molecular weight of about 88 kd. Deduced amino acid sequence, as provided in SEQ ID NO: 1, for one form of the molecule, yields a molecular weight of about 88 kd.

It should be noted that there is an apparent discrepancy in molecular weight in that the COS-1 isolate is glycosylated, while molecular weight from deduced amino acid sequences does not account for glycosylation. Membrane proteins are known to exhibit aberrant migration in gel systems, however, which may explain the difference observed here.

Also a part of the invention are chimeric and fusion proteins, which comprise a portion of FAPα which contain the molecule's catalytic domain, and additional, non FAPα components. The FAPα catalytic domain per se is also a part of the invention.

It is to be understood that, as described, FAPα may be glycosylated, with the type and amount of glycosylation varying, depending upon the type of cell expressing the molecule. The experiment described herein shows this. This is also true for the dimeric form of the molecule, first described herein, having a molecular weight of about 170 kilodaltons as determined by SDS-PAGE of unboiled samples.

The invention also comprehends the production of

expression vectors useful in producing the FAPα molecule. In their broadest aspect, these vectors comprise the entire FAPα coding sequence or portions thereof, operably linked to a promoter. Additional elements may be a part of the expression vector, such as protein domains fused to the FAPα protein or protein portions ("fusion protein") genes which confer antibiotic resistance, amplifiable genes, and so forth.

The coding sequences and vectors may also be used to prepare cell lines, wherein the coding sequence or expression vector is used to transfect or to transform a recipient host. The type of cell used may be prokaryotic, such as E. coli, or eukaryotes, such as yeast, CHO, COS, or other cell types.

The identification of nucleic acid molecules such as that set forth in SEQ ID NO: 1 also enables the artisan to identify and to isolate those nucleic acid molecules which hybridize to it under stringent conditions. "Stringent condition" as used herein, refers to those parameters set forth supra, whereby both murine and hamster sequences were also identified. It will be recognized by the skilled artisan that these conditions afford a degree of stringency which can be achieved using parameters which vary from those recited. Such variance is apprehended by the expression "stringent conditions".

The ability of nucleic acid molecules to hybridize to complementary molecules also enables the artisan to identify cells which express FAPα, via the use of a nucleic acid hybridization assay. One may use the sequences described in the invention to hybridize to complementary sequences, and thus identify them. In this way, one can target mRNA, e.g., which is present in any cell expressing the FAPα molecule.

It is of course understood that the nucleic acid molecules of the invention are also useful in the production of recombinant FAPα, in both monomeric and dimeric form. The examples clearly show that host cells are capable of assembling the dimeric forms. The recombinant protein may be used, e.g., as a source of an immunogen for generation of antibodies akin to known mAb F19, and with the same uses. Similarly, the recombinant protein, and/or cells which express

the molecule on their surface, may be used in assays to determine antagonists, agonists, or other molecules which interact with molecules having FAPα activity. Such substances may be, but are not necessarily limited to, substrates, inhibiting molecules, antibodies, and so forth. The molecules having FAPα activity may be, e.g., the monomeric or dimeric forms of FAPα, derivatives containing the catalytic domain, and so forth. The molecule having FAPα activity may be pure, or in the form of a cell extract, such as a transformed or transfected cell, which has received an FAPα active gene. Both prokaryotes and eukaryotes may be used. This last feature of the invention should be considered in light of the observed structural resemblances to membrane bound enzymes. This type of molecule is associated with certain properties which need not be described in detail here. It will suffice to say that inhibition or potentiation of these properties as associated with FAPα is a feature of this invention. For example, one may identify substrates or the substrate for FAPα molecules, via the use of recombinant cells or recombinant FAPα per se. The substrates can be modified to improve their effect, to lessen their effect, or simply to label them with detectable signals so that they can be used, e.g., to identify cells which express FAPα. Study of the interaction of substrate and FAPα, as well as that between FAPα and any molecule whatsoever, can be used to develop and/or to identify agonists and antagonists of the FAPα molecule.

Also a feature of the invention are isolated, dimeric FAPα

molecules which have a molecular weight of about 170 kilodaltons as determined by SDS-PAGE, their use as an enzymatic cleaving agent, and other uses as are described herein. Enzymatically active forms of FAPα may also be produced as recombinant fusion proteins, such as soluble fusion proteins comprising the catalytic domain of FAPα and other protein domains with suitable biochemical properties, including secretory signals, protease cleavage sites, tags for purification, and other elements known to the artisan.

Exemplary are CD8 peptide sequences, such as are described supra. The fact that FAPα has particular properties, as described herein, permits the identification of the molecule on cells expressing them. In turn, because the FAPα molecule is associated with tumors and tumor stromal cells, targeting of FAPα with therapeutic agents serves as a way to treat cancerous or precancerous condition, by administering sufficient therapeutic agent to alleviate cancer load.

The experiments showing the proteolytic properties of FAPα lead to yet a further aspect of the invention. It is well known that proteases which degrade extracellular matrix, or "ECM" proteins have an important role on certain aspects of tumor growth, including their effect on tumor cell invasion, tumor blood vessel formation (i.e., neoangiogenesis), and tumor metastasis. Collagens are of special interest vis-a-vis the substrates of proteases, as the collagens are an important part of the ECM. The fact that FAPα digests ECM suggests a therapeutic role for inhibitors of the molecule. "Inhibitors", as used herein, refers to molecules which interfere with FAPα enzyme function. Specifically excluded from such inhibitors is the monoclonal antibody F19. This mAb is known to bind to but not inhibit the enzyme function of FAPα, and hence it is not an inhibitor. The art is quite well versed with respect to monoclonal antibodies which both bind to and inhibit enzymes. Further examples of such inhibitors would include, e.g., substrate derivatives, such as modified collagen molecules, which interfere with the active site or sites of the FAPα molecule. Other suitable inhibitors will be apparent to the skilled artisan, and need not be listed here. In addition, the recombinant FAPα proteins and FAPα-transfected cell lines described supra can be employed in an enzymatic screening assay, using the substrate described supra or other suitable substrates, to identify inhibitors from any compound library. The identification of substances which interact with FAPα active molecules thereby leads to therapeutic treatment of conditions where a subject exhibit abnormal FAPα activity. Specifically, an amount of an

appropriate substance, be it an inhibitor (e.g, a collagen derivative, S-Valyl-pyrrolidine-2(R)-boronic acid), an agonist or an antogonist is administered to a subject in an amount sufficient to normalize FAPα activity.

Other aspects of the invention will be clear to the skilled artisan, and need not be set forth here.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

(1) GENERAL INFORMATION:

(i) APPLICANTS: Zimmermann, Rainer; Park, John E.; Rettig, Wolfgang; Old, Lloyd J.

(ii) TITLE OF INVENTION: ISOLATED DIMERIC FIBROBLAST ACTIVATION PROTEIN ALPHA, AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Felfe & Lynch

(B) STREET: 805 Third Avenue

(C) CITY: New York City

(D) STATE: New York

(E) COUNTRY: USA

(F) ZIP: 10022

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 3.5 inch, 2.0 MB storage

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: PC-DOS

(D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii)PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/619,280

(B) FILING DATE: 18-MARCH-1996

(C) CLASSIFICATION: 435

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/230,491

(B) FILING DATE: 20-APRIL-1994

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Hanson, Norman D.

(B) REGISTRATION NUMBER: 30,946

(C) REFERENCE/DOCKET NUMBER: LUD 5330.1-PCT

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (212) 688-9200

(B) TELEFAX: (212) 838-3884

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2815 Base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 1:

AAGAACGCCC CCAAAATCTG TTTCTAATTT TACAGAAATC TTTTGAAACT TGGCACGGTA 60
TTCAAAAGTC CGTGGAAAGA AAAAAACCTT GTCCTGGCTT CAGCTTCCAA CTACAAAGAC 120
AGACTTGGTC CTTTTCAACG GTTTTCACAG ATCCAGTGAC CCACGCTCTG AAGACAGAAT 180
TAGCTAACTT TCAAAAACAT CTGGAAAAAT GAAGACTTGG GTAAAAATCG TATTTGGAGT 240
TGCCACCTCT GCTGTGCTTG CCTTATTGGT GATGTGCATT GTCTTACGCC CTTCAAGAGT 300
TCATAACTCT GAAGAAAATA CAATGAGAGC ACTCACACTG AAGGATATTT TAAATGGAAC 360
ATTTTCTTAT AAAACATTTT TTCCAAACTG GATTTCAGGA CAAGAATATC TTCATCAATC 420
TGCAGATAAC AATATAGTAC TTTATAATAT TGAAACAGGA CAATCATATA CCATTTTGAG 480
TAATAGAACC ATGAAAAGTG TGAATGCTTC AAATTACGGC TTATCACCTG ATCGGCAATT 540
TGTATATCTA GAAAGTGATT ATTCAAAGCT TTGGAGATAC TCTTACACAG CAACATATTA 600
CATCTATGAC CTTAGCAATG GAGAATTTGT AAGAGGAAAT GAGCTTCCTC GTCCAATTCA 660
GTATTTATGC TGGTCGCCTG TTGGGAGTAA ATTAGCATAT GTCTATCAAA ACAATATCTA 720
TTTGAAACAA AGACCAGGAG ATCCACCTTT TCAAATAACA TTTAATGGAA GAGAAAATAA 780
AATATTTAAT GGAATCCCAG ACTGGGTTTA TGAAGAGGAA ATGCTTCCTA CAAAATATGC 840
TCTCTGGTGG TCTCCTAATG GAAAATTTTT GGCATATGCG GAATTTAATG ATAAGGATAT 900
ACCAGTTATT GCCTATTCCT ATTATGGCGA TGAACAATAT CCTAGAACAA TAAATATTCC 960
ATACCCAAAG GCTGGAGCTA AGAATCCCGT TGTTCGGATA TTTATTATCG ATACCACTTA 1020
CCCTGCGTAT GTAGGTCCCC AGGAAGTGCC TGTTCCAGCA ATGATAGCCT CAAGTGATTA 1080
TTATTTCAGT TGGCTCACGT GGGTTACTGA TGAACGAGTA TGTTTGCAGT GGCTAAAAAG 1140
AGTCCAGAAT GTTTCGGTCC TGTCTATATG TGACTTCAGG GAAGACTGGC AGACATGGGA 1200
TTGTCCAAAG ACCCAGGAGC ATATAGAAGA AAGCAGAACT GGATGGGCTG GTGGATTCTT 1260
TGTTTCAAGA CCAGTTTTCA GCTATGATGC CATTTCGTAC TACAAAATAT TTAGTGACAA 1320
GGATGGCTAC AAACATATTC ACTATATCAA AGACACTGTG GAAAATGCTA TTCAAATTAC 1380
AAGTGGCAAG TGGGAGGCCA TAAATATATT CAGAGTAACA CAGGATTCAC TGTTTTATTC 1440
TAGCAATGAA TTTGAAGAAT ACCCTGGAAG AAGAAACATC TACAGAATTA GCATTGGAAG 1500
CTATCCTCCA AGCAAGAAGT GTGTTACTTG CCATCTAAGG AAAGAAAGGT GCCAATATTA 1560
CACAGCAAGT TTCAGCGACT ACGCCAAGTA CTATGCACTT GTCTGCTACG GCCCAGGCAT 1620
CCCCATTTCC ACCCTTCATG ATGGACGCAC TGATCAAGAA ATTAAAATCC TGGAAGAAAA 1680

CAAGGAATTG GAAAATGCTT TGAAAAATAT CCAGCTGCCT AAAGAGGAAA TTAAGAAACT 1740
TGAAGTAGAT GAAATTACTT TATGGTACAA GATGATTCTT CCTCCTCAAT TTGACAGATC 1800
AAAGAAGTAT CCCTTGCTAA TTCAAGTGTA TGGTGGTCCC TGCAGTCAGA GTGTAAGGTC 1860
TGTATTTGCT GTTAATTGGA TATCTTATCT TGCAAGTAAG GAAGGGATGG TCATTGCCTT 1920
GGTGGATGGT CGAGGAACAG CTTTCCAAGG TGACAAACTC CTCTATGCAG TGTATCGAAA 1980
GCTGGGTGTT TATGAAGTTG AAGACCAGAT TACAGCTGTC AGAAAATTCA TAGAAATGGG 2040
TTTCATTGAT GAAAAAAGAA TAGCCATATG GGGCTGGTCC TATGGAGGAT ACGTTTCATC 2100
ACTGGCCCTT GCATCTGGAA CTGGTCTTTT CAAATGTGGT ATAGCAGTGG CTCCAGTCTC 2160
CAGCTGGGAA TATTACGCGT CTGTCTACAC AGAGAGATTC ATGGGTCTCC CAACAAAGGA 2220
TGATAATCTT GAGCACTATA AGAATTCAAC TGTGATGGCA AGAGCAGAAT ATTTCAGAAA 2280
TGTAGACTAT CTTCTCATCC ACGGAACAGC AGATGATAAT GTGCACTTTC AAAACTCAGC 2340
ACAGATTGCT AAAGCTCTGG TTAATGCACA AGTGGATTTC CAGGCAATGT GGTACTCTGA 2400
CCAGAACCAC GGCTTATCCG GCCTGTCCAC GAACCACTTA TACACCCACA TGACCCACTT 2460
CCTAAAGCAG TGTTTCTCTT TGTCAGACTA AAAACGATGC AGATGCAAGC CTGTATCAGA 2520
ATCTGAAAAC CTTATATAAA CCCCTCAGAC AGTTTGCTTA TTTTATTTTT TATGTTGTAA 2580
AATGCTAGTA TAAACAAACA AATTAATGTT GTTCTAAAGG CTGTTAAAAA AAAGATGAGG 2640
ACTCAGAAGT TCAAGCTAAA TATTGTTTAC ATTTTCTGGT ACTCTGTGAA AGAAGAGAAA 2700
AGGGAGTCAT GCATTTTGCT TTGGACACAG TGTTTTATCA CCTGTTCATT TGAAGAAAAA 2760
TAATAAAGTC AGAAGTTCAA AAAAAAAAAA AAAAAAAAAA AAAGCGGCCG CTCGA 2815

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 760 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Lys Thr Trp Val Lys Ile Val Phe Gly Val Ala Thr Ser Ala Val
5 10 15
Leu Ala Leu Leu Val Met Cys Ile Val Leu Arg Pro Ser Arg Val His
20 25 30
Asn Ser Glu Glu Asn Thr Met Arg Ala Leu Thr Leu Lys Asp Ile Leu
35 40 45
Asn Gly Thr Phe Ser Tyr Lys Thr Phe Phe Pro Asn Trp Ile Ser Gly

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     50                  55                  60
Gln Glu Tyr Leu His Gln Ser Ala Asp Asn Asn Ile Val Leu Tyr Asn
 65                  70                  75                  80
Ile Glu Thr Gly Gln Ser Tyr Thr Ile Leu Ser Asn Arg Thr Met Lys
                 85                  90                  95
Ser Val Asn Ala Ser Asn Tyr Gly Leu Ser Pro Asp Arg Gln Phe Val
            100                 105                 110
Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp Arg Tyr Ser Tyr Thr Ala
        115                 120                 125
Thr Tyr Tyr Ile Tyr Asp Leu Ser Asn Gly Glu Phe Val Arg Gly Asn
    130                 135                 140
Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys Trp Ser Pro Val Gly Ser
145                 150                 155                 160
Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile Tyr Leu Lys Gln Arg Pro
                165                 170                 175
Gly Asp Pro Pro Phe Gln Ile Thr Phe Asn Gly Arg Glu Asn Lys Ile
            180                 185                 190
Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu Glu Glu Met Leu Pro Thr
        195                 200                 205
Lys Tyr Ala Leu Trp Trp Ser Pro Asn Gly Lys Phe Leu Ala Tyr Ala
    210                 215                 220
Glu Phe Asn Asp Lys Asp Ile Pro Val Ile Ala Tyr Ser Tyr Tyr Gly
225                 230                 235                 240
Asp Glu Gln Tyr Pro Arg Thr Ile Asn Ile Pro Tyr Pro Lys Ala Gly
                245                 250                 255
Ala Lys Asn Pro Val Val Arg Ile Phe Ile Ile Asp Thr Thr Tyr Pro
            260                 265                 270
Ala Tyr Val Gly Pro Gln Glu Val Pro Val Pro Ala Met Ile Ala Ser
        275                 280                 285
Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp Val Thr Asp Glu Arg Val
    290                 295                 300
Cys Leu Gln Trp Leu Lys Arg Val Gln Asn Val Ser Val Leu Ser Ile
305                 310                 315                 320
Cys Asp Phe Arg Glu Asp Trp Gln Thr Trp Asp Cys Pro Lys Thr Gln
                325                 330                 335
Glu His Ile Glu Glu Ser Arg Thr Gly Trp Ala Gly Gly Phe Phe Val
            340                 345                 350
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Ser Arg Pro Val Phe Ser Tyr Asp Ala Ile Ser Tyr Tyr Lys Ile Phe
355 360 365
Ser Asp Lys Asp Gly Tyr Lys His Ile His Tyr Ile Lys Asp Thr Val
370 375 380
Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys Trp Glu Ala Ile Asn Ile
385 390 395 400
Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr Ser Ser Asn Glu Phe Glu
405 410 415
Glu Tyr Pro Gly Arg Arg Asn Ile Tyr Arg Ile Ser Ile Gly Ser Tyr
420 425 430
Pro Pro Ser Lys Lys Cys Val Thr Cys His Leu Arg Lys Glu Arg Cys
435 440 445
Gln Tyr Tyr Thr Ala Ser Phe Ser Asp Tyr Ala Lys Tyr Tyr Ala Leu
450 455 460
Val Cys Tyr Gly Pro Gly Ile Pro Ile Ser Thr Leu His Asp Gly Arg
465 470 475 480
Thr Asp Gln Glu Ile Lys Ile Leu Glu Glu Asn Lys Glu Leu Glu Asn
485 490 495
Ala Leu Lys Asn Ile Gln Leu Pro Lys Glu Glu Ile Lys Lys Leu Glu
500 505 510
Val Asp Glu Ile Thr Leu Trp Tyr Lys Met Ile Leu Pro Pro Gln Phe
515 520 525
Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile Gln Val Tyr Gly Gly Pro
530 535 540
Cys Ser Gln Ser Val Arg Ser Val Phe Ala Val Asn Trp Ile Ser Tyr
545 550 555 560
Leu Ala Ser Lys Glu Gly Met Val Ile Ala Leu Val Asp Gly Arg Gly
565 570 575
Thr Ala Phe Gln Gly Asp Lys Leu Leu Tyr Ala Val Tyr Arg Lys Leu
580 585 590
Gly Val Tyr Glu Val Glu Asp Gln Ile Thr Ala Val Arg Lys Phe Ile
595 600 605
Glu Met Gly Phe Ile Asp Glu Lys Arg Ile Ala Ile Trp Gly Trp Ser
610 615 620
Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu Ala Ser Gly Thr Gly Leu
625 630 635 640
Phe Lys Cys Gly Ile Ala Val Ala Pro Val Ser Ser Trp Glu Tyr Tyr

645 650 655

Ala Ser Val Tyr Thr Glu Arg Phe Met Gly Leu Pro Thr Lys Asp Asp

660 665 670

Asn Leu Glu His Tyr Lys Asn Ser Thr Val Met Ala Arg Ala Glu Tyr

675 680 685

Phe Arg Asn Val Asp Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn

690 695 700

Val His Phe Gln Asn Ser Ala Gln Ile Ala Lys Ala Leu Val Asn Ala

705 710 715 720

Gln Val Asp Phe Gln Ala Met Trp Tyr Ser Asp Gln Asn His Gly Leu

725 730 735

Ser Gly Leu Ser Thr Asn His Leu Tyr Thr His Met Thr His Phe Leu

740 745 750

Lys Gln Cys Phe Ser Leu Ser Asp

755 760

We claim:

1. Isolated, dimeric FAPα molecule, having a molecular weight of about 170 kilodaltons as determined by SDS-PAGE, wherein said dimeric FAPα molecule is capable of degrading extracellular matrix proteins.

2. The dimeric FAPα molecule of claim 1, wherein each monomer of said dimeric FAPα molecule consists of the amino acid sequence of SEQ ID NO: 2.

3. The dimeric FAPα molecule of claim 1, produced recombinantly.

4. The dimeric FAPα molecule of claim 3, produced by a eukaryotic cell.

5. Isolated protein consisting of:
   (i) the FAPα catalytic domain, and
   (ii) at least one portion of a non FAPα protein.

6. Method for cleaving a terminal dipeptide of formula Xaa-Pro from a molecule, comprising contacting said molecule with a second molecule, said second molecule having FAPα enzymatic activity.

7. The method of claim 6, wherein said second molecule is isolated, dimeric FAPα.

8. The method of claim 6, wherein said second molecule comprises an FAPα catalytic domain.

9. Method for identifying a substance which interacts with a molecule having FAPα activity, comprising combining said molecule with a sample to be tested, and determining any interaction with said molecule as an indication of a molecule which interacts with a molecule having FAPα activity.

10. The method of claim 9, wherein said FAPα molecule is dimeric.

11. The method of claim 9, wherein said molecule comprises a FAPα catalytic domain.

12. The method of claim 9, wherein said substance is an antagonist of FAPα activity.

13. The method of claim 9, wherein said substance is an agonist of FAPα activity.

14. The method of claim 9, wherein said substance is an inhibitor of FAPα activity.

15. The method of claim 9, comprising combining said substance with a cell extract which has FAPα activity.

16. The method of claim 15, wherein said cell extract is an extract of a cell which has been transformed or transfected with a nucleic acid molecule which encodes a molecule with FAPα activity.

17. The method of claim 16, wherein said cell is a

prokaryote.

18. The method of claim 16, wherein said cell is a eukaryote.

19. Method for treating a subject with a pathological condition characterized by abnormal FAPα activity, comprising administering to a subject in need thereof an amount of a substance which interacts with molecules having FAPα activity sufficient to normalize the FAPα activity level in said subject.

20. The method of claim 19, comprising administering an inhibitor of FAPα activity.

21. The method of claim 20, wherein said inhibitor is a collagen derivative.

22. The method of claim 20, wherein said inhibitor is (S) -Valyl-pyrrolidine-2(R) -boronic acid.

23. The method of claim 19, wherein said substance is an agonist of FAPα activity.

24. The method of claim 19, wherein said substance is an antagonist of FAPα activity.

25. Method for determining if a substance interacts with a molecule having FAPα activity, comprising combining said substance and said molecule with Ala-Pro-AFC, determining interaction of said molecule with Ala-Pro-AFC, and comparing said interaction to interaction of said molecule with Ala-Pro-AFC in the absence of said substance, wherein a difference therebetween indicate that said substance interactions with said molecule.

26. Fusion protein comprising a portion of an FAPα molecule sufficient to retain FAPα activity and a non FAPα amino

acid sequence, wherein said fusion protein is water soluble.

27. The fusion protein of claim 26, wherein said non FAPα amino acid sequence is an amino acid sequence found in a CD8 protein.

28. The fusion protein of claim 27, wherein said CD8 protein is a murine protein.

29. The fusion protein of claim 27, wherein said CD8 protein is a human protein.

30. The fusion protein of claim 27 comprising amino acid 1 to 189 of murine CD8 linked to amino acids 27 through 760 of FAPα.