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(54) **NUCLEIC ACID MOLECULES ENCODING  
TRANSMEMBRANE SERINE PROTEASES,  
THE ENCODED PROTEINS AND METHODS  
BASED THEREON**

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435/69.1; 435/320.1; 435/325

(57) **ABSTRACT**

Provided herein is are polypeptides that include the protease  
domain of a type II transmembrane serine protease (MTSP)  
as a single chain. Methods using the polypeptides to identify  
compounds that modulate the protease activity of an MTSP  
are provided. Also provided are MTSPs designated MTSP3  
and MTSP4 and a form of an MTSP designated MTSP6.

### Domain organization of MTSP3

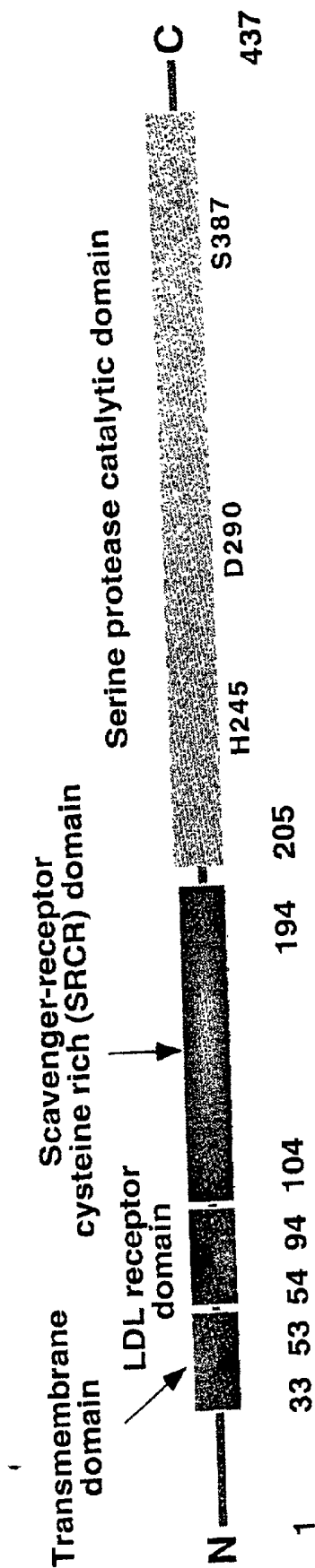


FIGURE 1

### SPLICE VARIANT AND DOMAIN ORGANIZATION OF MTSP4

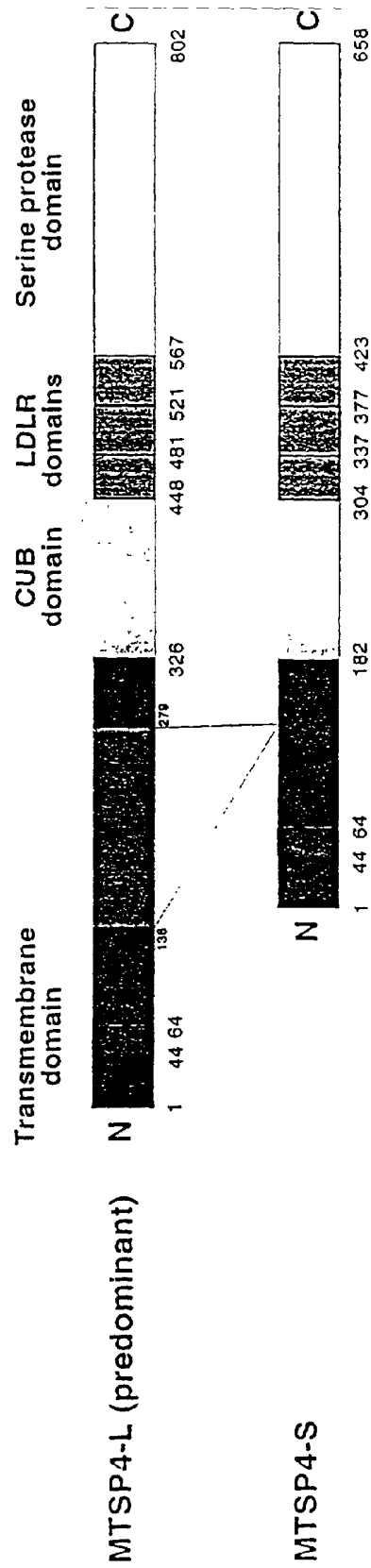


FIGURE 2

# DOMAIN ORGANIZATION OF HUMAN MTSP6

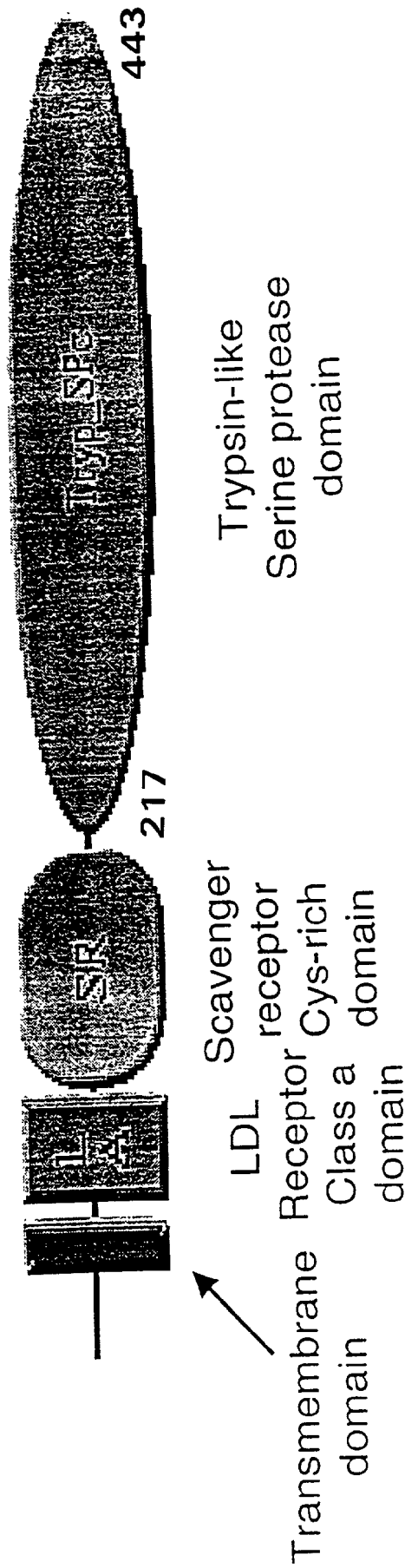


FIGURE 3

MTSP3	194	LACGKS-----LKT <sup>▽</sup> PRVVGGEASVDSWPWQVSIQYDKQHVC <sup>205</sup> CGSILD	236
MTSP4-S	396	PQCDGRPDCRDGSD <sup>205</sup> EFHICECGLQGFSSRIVGGAVSSEGEWPWQASLQVRGRHICGGALIA	455
MTSP4-L	540	PQCDGRPDCRDGSD <sup>424</sup> EFHICECGLQGFSSRIVGGAVSSEGEWPWQASLQVRGRHICGGALIA	599
MTSP6	205	TACGHR-----RGYSSRIVCGNMSLLSQWPWQASLQFQGYHL <sup>568</sup> CGGSVIT	248
			217 +
MTSP3	237	PHWVLTAAHCFR <sup>205</sup> KIIFDVFN--WKVRAGSDKLG <sup>205</sup> S----FPSLAVAKITIIIEFNPMYPKDND	290
MTSP4-S	456	DRWVITA <sup>205</sup> AHCFQEDSMAS <sup>205</sup> TVLWTVFLGK <sup>205</sup> VWQNSRW <sup>205</sup> PGEV <sup>205</sup> SFKV <sup>205</sup> SRL <sup>205</sup> LLHPY <sup>205</sup> HEEDS <sup>205</sup> HDYD	515
MTSP4-L	600	DRWVITA <sup>205</sup> AHCFQEDSMAS <sup>205</sup> TVLWTVFLGK <sup>205</sup> VWQNSRW <sup>205</sup> PGEV <sup>205</sup> SFKV <sup>205</sup> SRL <sup>205</sup> LLHPY <sup>205</sup> HEEDS <sup>205</sup> HDYD	659
MTSP6	249	PLWIT <sup>205</sup> AHCVYDLYLPKS--WTIQVGLVSL <sup>205</sup> LD--NPAPSHLVEK <sup>205</sup> IVYHSKYKPKRLGND	304
MTSP3	291	I <sup>205</sup> ALMKLQFPLTFSGTVR <sup>205</sup> PICLPFFDEE <sup>205</sup> ITPATPLWII <sup>205</sup> GWGFTKQNGGKMSDILLQASVQV	350
MTSP4-S	516	VALLQLDHPV <sup>205</sup> VRSAAVR <sup>205</sup> PVCLPARSH <sup>205</sup> FFEPGLHCWITG <sup>205</sup> WGALRE-GGPTSNALQKVDVQL	574
MTSP4-L	660	VALLQLDHPV <sup>205</sup> VRSAAVR <sup>205</sup> PVCLPARSH <sup>205</sup> FFEPGLHCWITG <sup>205</sup> WGALRE-GGPTSNALQKVDVQL	718
MTSP6	305	I <sup>205</sup> ALMKLAGPLTFNEMIQPVCLPNSEENFPD <sup>205</sup> GKVCWTS <sup>205</sup> SGWATED-GCDAS <sup>205</sup> PVLNHA <sup>205</sup> AVPL	363
MTSP3	351	UDSTRCNAD <sup>205</sup> DAYQGEVTEK <sup>205</sup> MMCAGIPEGGV <sup>205</sup> DTCCGDSGG <sup>205</sup> PLMYQSDQ--WHVVGIVSWGY	408
MTSP4-S	575	IPQDLCS--EVYRYQV <sup>205</sup> TPRMLCAGYR <sup>205</sup> KGKKDACQ <sup>205</sup> GDSGG <sup>205</sup> PLVCKALSGRW <sup>205</sup> FLAGL <sup>205</sup> VS <sup>205</sup> WGL	632
MTSP4-L	719	IPQDLCS--EVYRYQV <sup>205</sup> TPRMLCAGYR <sup>205</sup> KGKKDACQ <sup>205</sup> GDSGG <sup>205</sup> PLVCKALSGRW <sup>205</sup> FLAGL <sup>205</sup> VS <sup>205</sup> WGL	776
MTSP6	364	ISNKICNHRD <sup>205</sup> VYGGIIS <sup>205</sup> PSMLCAGYLT <sup>205</sup> GGVDS <sup>205</sup> CCGDSGG <sup>205</sup> PLVCQERR-LWKL <sup>205</sup> VGAT <sup>205</sup> SFGI	422
MTSP3	409	GCGGPST <sup>205</sup> FGVYTKV <sup>205</sup> SAYLNWIYNV <sup>205</sup> WKAEL	437
MTSP4-S	533	GCGRPNY <sup>205</sup> FGVYTRITG <sup>205</sup> VISWI <sup>205</sup> QQVVT	658
MTSP4-L	777	GCGRPNY <sup>205</sup> FGVYTRITG <sup>205</sup> VISWI <sup>205</sup> QQVVT	802
MTSP6	423	GCAEVN <sup>205</sup> KPGVYTRVTS <sup>205</sup> FLDWIHEQ <sup>205</sup> MERDLKT	453

▽ cleavage site

+ potential glycosylation site

\* unpaired cysteine

FIGURE 4

Domain organization of MTSP3

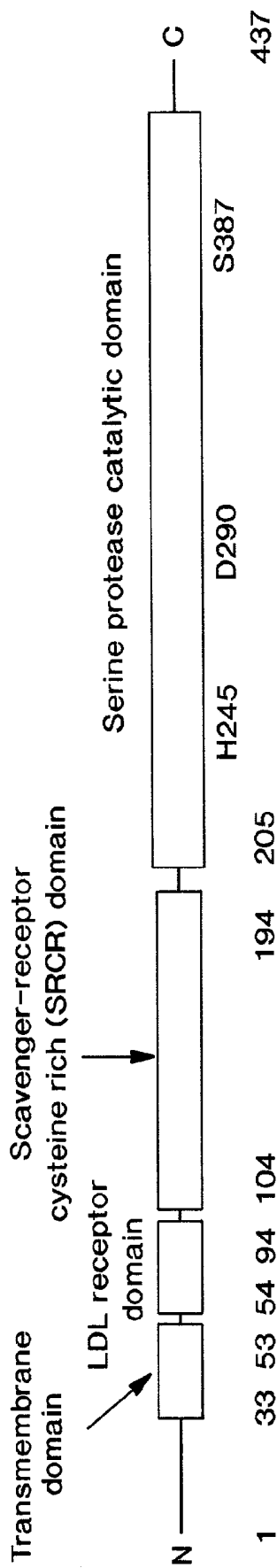


FIG. 1

Splice variant and domain organization of MTSP4

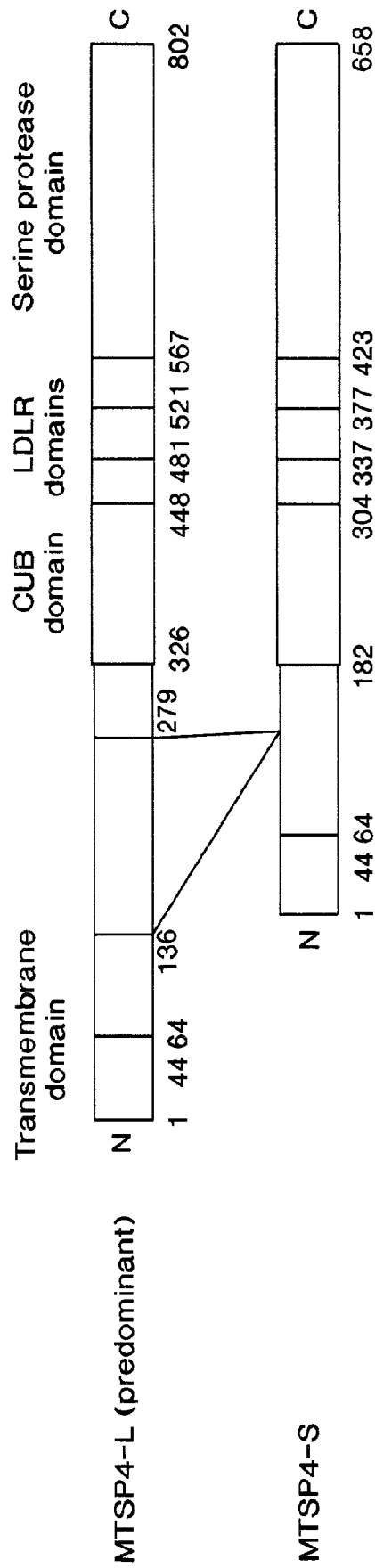


FIG. 2

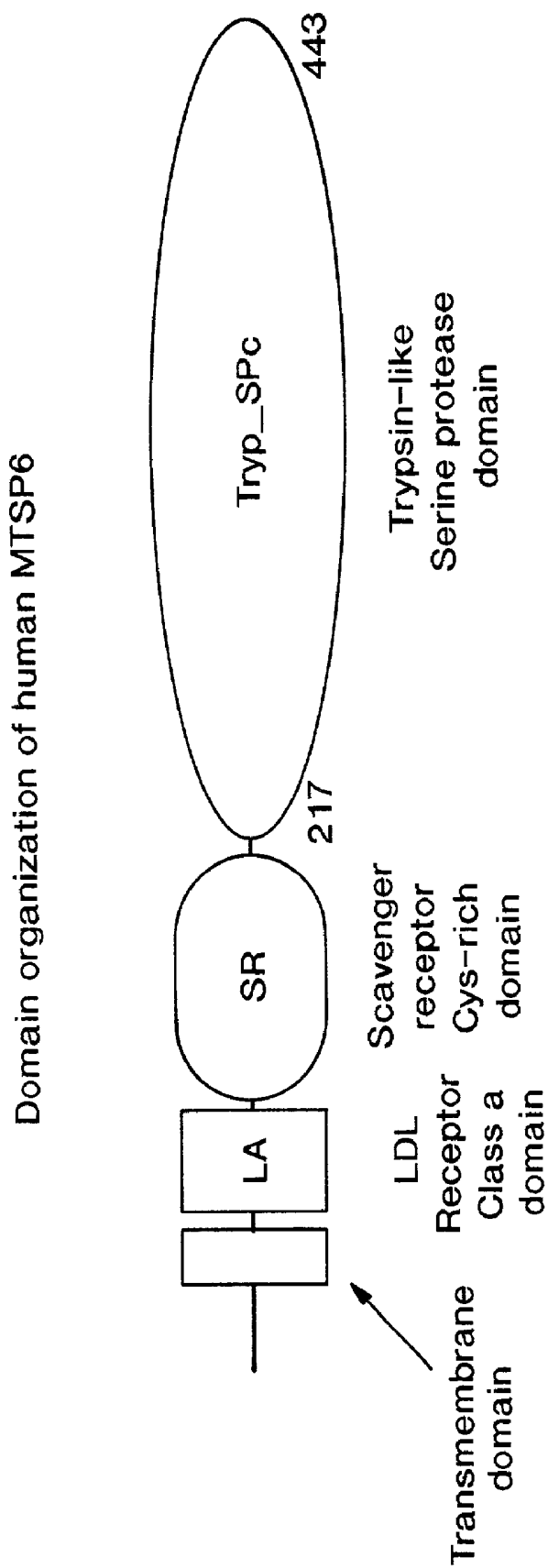


FIG. 3

			▽		
MTSP3	194	LACGKS-----LKT	PRVVGEEASVDSWPQVSIQYDKQHVC	CGGSILD 236	
			205		
MTSP4-S	396	PQCDGRPDCRDGSDEEHCECGLQG	PSSRIVGGAVSSEGEWPQASLQVRGR	HICGGALIA 455	
			424		
MTSP4-L	540	PQCDGRPDCRDGSDEEHCECGLQG	PSSRIVGGAVSSEGEWPQASLQVRGR	HICGGALIA 599	
			568		
MTSP6	205	TACGHR-----RGYSSRIVGGN	MSLLSQWPQASLQFQGYHL	CGGSVIT 248	
			217 +		
MTSP3	237	PHWVLTAAHCFRKHTDVFN--WKV	RAGSDKLGSD---P	PSLAVAKIIIEFNPMYPKDND 290	
MTSP4-S	456	DRWVITAACHCFQEDSMAS	TVLWTVFLGKQNSRWPGEV	SFKVSRLLLHPYHEEDSHDYD 515	
MTSP4-L	600	DRWVITAACHCFQEDSMAS	TVLWTVFLGKQNSRWPGEV	SFKVSRLLLHPYHEEDSHDYD 718	
MTSP6	249	PLWIITAACHCVYDLYLPKS--	WTIQVGLVSLLD--	NPAPSHLVEKIVYHSKYKPKRLGND 304	
MTSP3	291	IALMKLQFPLTFSGTVR	PICLPFFDEELTPATPLW	IIGWFTKQNGGKMSDILLQASVQV 350	
			*		
MTSP4-S	516	VALLQLDHPVVRSAAVR	PVCLPARSHFFEPGLHC	WITGWALRE-GGPISNALQKVDVQL 574	
			*		
MTSP4-L	660	VALLQLDHPVVRSAAVR	PVCLPARSHFFEPGLHC	WITGWALRE-GGPISNALQKVDVQL 718	
			*		
MTSP6	305	IALMKLAGPLTFNEMI	QPVCLPNSSEENFPD	GKVCWTSGWGATED-GGDASPVLNHAAVPL 363	
			*		
MTSP3	351	IDSTRCNADDAYQGEVTE	KMMCAGIPEGGVDT	CQGDSCGGPLMYQSDQ--WHVVGVISWGY 408	
MTSP4-S	575	IPQDLCS--EVYRYQV	TPRMLCAGYRKGK	DACQGDSCGGPLVCKALSGRWF	LAGLVS
MTSP4-L	719	IPQDLCS--EVYRYQV	TPRMLCAGYRKGK	DACQGDSCGGPLVCKALSGRWF	LAGLVS
MTSP6	364	ISNKICNHRDVYGGI	IISPSMLCAGYLTGG	VDSQGDSCGGPLVCQERR-LWKVL	VGATSF
MTSP3	409	GCGGPSTPGVYTKV	SAYLNWIYNVWKAEL	437	
MTSP4-S	633	GCGRPNYFGVYTRIT	GVISWIQQVVT	658	
MTSP4-L	777	GCGRPNYFGVYTRIT	GVISWIQQVVT	802	
MTSP6	423	GCAEVNKP	GVYTRVTSFLDWIHEQ	MERDLKT 453	

▽ cleavage site  
 + potential glycosylation site  
 \* unpaired cysteine

FIG. 4

**NUCLEIC ACID MOLECULES ENCODING  
TRANSMEMBRANE SERINE PROTEASES, THE  
ENCODED PROTEINS AND METHODS BASED  
THEREON**

RELATED APPLICATIONS

[0001] For U.S. purposes, benefit of priority under 35 U.S.C. § 119(e) to U.S. provisional application Serial No. 60/179,982, to Edwin L. Madison and Edgar O. Ong, filed Feb. 3, 2000, entitled "NUCLEOTIDE AND PROTEIN SEQUENCES OF A TRANSMEMBRANE SERINE PROTEASE AND METHODS BASED THEREOF"; to U.S. provisional application Serial No. 60/183,542, to Edwin L. Madison and Edgar O. Ong, filed Feb. 18, 2000, entitled "NUCLEOTIDE AND PROTEIN SEQUENCES OF A TRANSMEMBRANE SERINE PROTEASE AND METHODS BASED THEREOF"; to U.S. provisional application Serial No. 60/213,124, to Edwin L. Madison and Edgar O. Ong, filed Jun. 22, 2000, entitled "NUCLEOTIDE AND PROTEIN SEQUENCES OF A TRANSMEMBRANE SERINE PROTEASE AND METHODS BASED THEREOF"; to U.S. provisional application Serial No. 60/220,970, to Edwin L. Madison and Edgar O. Ong, filed Jul. 26, 2000, entitled "NUCLEOTIDE AND PROTEIN SEQUENCES OF A TRANSMEMBRANE SERINE PROTEASE AND METHODS BASED THEREOF"; and to U.S. provisional application Serial No. 06/234,840 to Edwin L. Madison, Edgar O. Ong and Jiunn-Chern Yeh, filed Sep. 22, 2000, entitled "NUCLEIC ACID MOLECULES ENCODING TRANSMEMBRANE SERINE PROTEASES, THE ENCODED PROTEINS AND METHODS BASED THEREON" is claimed herein. Benefit of priority under 35 U.S.C. §120 to U.S. application Ser. No. 09/657,968, to Edwin L. Madison, Joseph Edward Semple, Gary Samuel Coombs, John Eugene Reiner, Edgar O. Ong, Gian Luca Araldi, filed Sep. 8, 2000, entitled "INHIBITORS OF SERINE PROTEASE ACTIVITY OF MATRIPTASE OR MTSP1" is also claimed herein. This application is a continuation-in-part of U.S. application Ser. No. 09/657,986. For international purposes, benefit of priority to each of the above-noted applications is claimed herein.

[0002] This application is related to U.S. provisional application Serial No. 60/166,391 to Edwin L. Madison and Edgar O. Ong, filed Nov. 18, 1999 entitled "NUCLEOTIDE AND PROTEIN SEQUENCES OF PROTEASE DOMAINS OF ENDOTHELIASE AND METHODS BASED THEREON". This application is also related to International PCT application No. PCT/US00/31803, filed Nov. 17, 2000.

[0003] The above-noted provisional applications, patent application and International PCT application are incorporated by reference in their entirety. All patents, applications, published applications and other publications and sequences from GenBank and other data bases referred to herein are incorporated by reference in their entirety.

FIELD OF INVENTION

[0004] Nucleic acid molecules that encode proteases and portions thereof, particularly protease domains are provided. Also provided are prognostic, diagnostic and therapeutic methods using the proteases and domains thereof and the encoding nucleic acid molecules.

BACKGROUND OF THE INVENTION AND  
OBJECTS THEREOF

[0005] Cancer a leading cause of death in the United States, developing in one in three Americans; one of every four Americans dies of cancer. Cancer is characterized by an increase in the number of abnormal neoplastic cells, which proliferate to form a tumor mass, the invasion of adjacent tissues by these neoplastic tumor cells, and the generation of malignant cells that metastasize via the blood or lymphatic system to regional lymph nodes and to distant sites.

[0006] Among the hallmarks of cancer is a breakdown in the communication among tumor cells and their environment. Normal cells do not divide in the absence of stimulatory signals, cease dividing in the presence of inhibitory signals. Growth-stimulatory and growth-inhibitory signals, are routinely exchanged between cells within a tissue. In a cancerous, or neoplastic, state, a cell acquires the ability to "override" these signals and to proliferate under conditions in which normal cells do not grow.

[0007] In order to proliferate tumor cells acquire a number of distinct aberrant traits reflecting genetic alterations. The genomes of certain well-studied tumors carry several different independently altered genes, including activated oncogenes and inactivated tumor suppressor genes. Each of these genetic changes appears to be responsible for imparting some of the traits that, in the aggregate, represent the full neoplastic phenotype.

[0008] A variety of biochemical factors have been associated with different phases of metastasis. Cell surface receptors for collagen, glycoproteins such as laminin, and proteoglycans, facilitate tumor cell attachment, an important step in invasion and metastases. Attachment triggers the release of degradative enzymes which facilitate the penetration of tumor cells through tissue barriers. Once the tumor cells have entered the target tissue, specific growth factors are required for further proliferation. Tumor invasion (or progression) involves a complex series of events, in which tumor cells detach from the primary tumor, break down the normal tissue surrounding it, and migrate into a blood or lymphatic vessel to be carried to a distant site. The breaking down of normal tissue barriers is accomplished by the elaboration of specific enzymes that degrade the proteins of the extracellular matrix that make up basement membranes and stromal components of tissues.

[0009] A class of extracellular matrix degrading enzymes have been implicated in tumor invasion. Among these are the matrix metalloproteinases (MMP). For example, the production of the matrix metalloproteinase stromelysin is associated with malignant tumors with metastatic potential (see, e.g., McDonnell et al. (1990) *Smnrs. in Cancer Biology* 1:107-115; McDonnell et al. (1990) *Cancer and Metastasis Reviews* 9:309-319).

[0010] The capacity of cancer cells to metastasize and invade tissue is facilitated by degradation of the basement membrane. Several proteinase enzymes, including the MMPs, have been reported to facilitate the process of invasion of tumor cells. MMPs are reported to enhance degradation of the basement membrane, which thereby permits tumorous cells to invade tissues. For example, two major metalloproteinases having molecular weights of about 70 kDa and 92 kDa appear to enhance ability of tumor cells to metastasize.

[0011] Type II Transmembrane Serine Proteases (TTSPs)

[0012] In addition to the MMPs, serine proteases have been implicated in neoplastic disease progression. Most serine proteases, which are either secreted enzymes or are sequestered in cytoplasmic storage organelles, have roles in blood coagulation, wound healing, digestion, immune responses and tumor invasion and metastasis. A class cell surface proteins designated type II transmembrane serine proteases, which are membrane-anchored proteins with N-terminal extracellular domains, has been identified. As cell surface proteins, they are positioned to play a role in intracellular signal transduction and in mediating cell surface proteolytic events.

[0013] Cell surface proteolysis is a mechanism for the generation of biologically active proteins that mediate a variety of cellular functions. These membrane-anchored proteins, include a disintegrin-like and metalloproteinase (ADAM) and membrane-type matrix metalloproteinase (MT-MMP). In mammals, at least 17 members of the family are known, including seven in humans (see, Hooper et al. (2001) *J. Biol. Chem.* 276:857-860). These include: corin (accession nos. AF133845 and AB013874; see, Yan et al. (1999) *J. Biol. Chem.* 274:14926-14938; Tomia et al. (1998) *J. Biochem.* 124:784-789; Uan et al. (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97:8525-8529); enterpeptidase (also designated enterokinase; accession no. U09860 for the human protein; see, Kitamoto et al. (1995) *Biochem.* 27: 4562-4568; Yahagi et al. (1996) *Biochem. Biophys. Res. Commun.* 219:806-812; Kitamoto et al. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91:7588-7592; Matsushima et al. (1994) *J. Biol. Chem.* 269:19976-19982); human airway trypsin-like protease (HAT; accession no. AB002134; see Yamaoka et al. *J. Biol. Chem.* 273:11894-11901); MTSP1 and matriptase (also called TADG-15; see SEQ ID Nos. 1 and 2; accession nos. AF133086/AF118224, AF04280022; Takeuchi et al. (1999) *Proc. Natl. Acad. Sci. U.S.A.* 96:11054-1161; Lin et al. (1999) *J. Biol. Chem.* 274:18231-18236; Takeuchi et al. (2000) *J. Biol. Chem.* 275:26333-26342; and Kim et al. (1999) *Immunogenetics* 49:420-429); hepsin (see, accession nos. M18930, AF030065, X70900; Leytus et al. (1988) *Biochem.* 27: 11895-11901; Vu et al. (1997) *J. Biol. Chem.* 272:31315-31320; and Farley et al. (1993) *Biochem. Biophys. Acta* 1173:350-352; and see, U.S. Pat. No. 5,972,616); TMPRSS2 (see, Accession Nos. U75329 and AF113596; Paoloni-Giacobino et al. (1997) *Genomics* 44:309-320; and Jacquinet et al. (2000) *FEBS Lett.* 468: 93-100); and TMPRSS4 (see, Accession No. NM 016425; Wallrapp et al. (2000) *Cancer* 60:2602-2606).

[0014] Serine proteases, including transmembrane serine proteases, have been implicated in processes involved in neoplastic development and progression. While the precise role of these proteases has not been elaborated, serine proteases and inhibitors thereof are involved in the control of many intra- and extracellular physiological processes, including degradative actions in cancer cell invasion, metastatic spread, and neovascularization of tumors, that are involved in tumor progression. It is believed that proteases are involved in the degradation of extracellular matrix (ECM) and contribute to tissue remodeling, and are necessary for cancer invasion and metastasis. The activity and/or expression of some proteases have been shown to correlate with tumor progression and development.

[0015] For example, a membrane-type serine protease MTSP1 (also called matriptase; see SEQ ID Nos. 1 and 2 from U.S. Pat. No. 5,972,616; and GenBank Accession No. AF118224; (1999) *J. Biol. Chem.* 274:18231-18236; U.S. Pat. No. 5,972,616; see, also Takeuchi (1999) *Proc. Natl. Acad. Sci. U.S.A.* 96:11054-1161) that is expressed in epithelial cancer and normal tissue (Takeuchi et al. (1999) *Proc. Natl. Acad. Sci. USA*, 96(20):11054-61) has been identified. Matriptase was originally identified in human breast cancer cells as a major gelatinase (see, U.S. Pat. No. 5,482,848), a type of matrix metalloproteinase (MMP). It has been proposed that it plays a role in the metastasis of breast cancer. Its primary cleavage specificity is Arg-Lys residues. Matriptase also is expressed in a variety of epithelial tissues with high levels of activity and/or expression in the human gastrointestinal tract and the prostate.

[0016] Prostate-specific antigen (PSA), a kallikrein-like serine protease, degrades extracellular matrix glycoproteins fibronectin and laminin, and, has been postulated to facilitate invasion by prostate cancer cells (Webber et al. (1995) *Clin. Cancer Res.*, 1(10):1089-94). Blocking PSA proteolytic activity with PSA-specific monoclonal antibodies results in a dose-dependent decrease in vitro in the invasion of the reconstituted basement membrane Matrigel by LNCaP human prostate carcinoma cells which secrete high levels of PSA.

[0017] Hepsin, a cell surface serine protease identified in hepatoma cells, is overexpressed in ovarian cancer (Tanimoto et al. (1997) *Cancer Res.*, 57(14):2884-7). The hepsin transcript appears to be abundant in carcinoma tissue and is almost never expressed in normal adult tissue, including normal ovary. It has been suggested that hepsin is frequently overexpressed in ovarian tumors and therefore may be a candidate protease in the invasive process and growth capacity of ovarian tumor cells.

[0018] A serine protease-like gene, designated normal epithelial cell-specific 1 (NES1) (Liu et al., *Cancer Res.*, 56(14):3371-9 (1996)) has been identified. Although expression of the NES1 mRNA is observed in all normal and immortalized nontumorigenic epithelial cell lines, the majority of human breast cancer cell lines show a drastic reduction or a complete lack of its expression. The structural similarity of NES1 to polypeptides known to regulate growth factor activity and a negative correlation of NES1 expression with breast oncogenesis suggest a direct or indirect role for this protease-like gene product in the suppression of tumorigenesis.

[0019] Hence transmembrane serine proteases appear to be involved in the etiology and pathogenesis of tumors. There is a need to further elucidate their role in these processes and to identify additional transmembrane proteases. Therefore, it is an object herein to provide transmembrane serine protease (MTSP) proteins and nucleic acids encoding such MTSP proteases that are involved in the regulation of or participate in tumorigenesis and/or carcinogenesis. It is also an object herein to provide prognostic, diagnostic, therapeutic screening methods using the such proteases and the nucleic acids encoding such proteases.

#### SUMMARY OF THE INVENTION

[0020] Provided herein are isolated protease domains of the Transmembrane Serine Protease family, particularly the

Type II Transmembrane Serine Protease (TTSP) family (also referred to herein as MTSPs), and more particularly TTSP family members whose functional activity differs in tumor cells from non-tumor cells in the same tissue. For example, the MTSPs include those that are activated and/or expressed in tumor cells at different levels, typically higher, from non-tumor cells; and those from cells in which substrates therefor differ in tumor cells from non-tumor cells or otherwise alter the specificity of the MTSP.

**[0021]** The MTSP family as intended herein does not include any membrane anchored or spanning proteases that are expressed on endothelial cells. Included among the MTSPs are several heretofore unidentified MTSP family members, designated herein as MTSP3 and MTSP4 and a new form of a protein designated herein as MTSP6. In addition to the protease domains of each of MTSP3 and MTSP4, the full-length proteins, including those that results from splice variants, zymogens and activated forms, and uses thereof, are also provided.

**[0022]** The protease domains as provided herein are single-chain polypeptides, with an N-terminus (such as IV, VV, IL and II) generated at the cleavage site (generally having the consensus sequence R↓VVGG, R↓IVGG, R↓IVNG, R↓ILGG, R↓VGLL, R↓ILGG or a variation thereof; an N-terminus R↓V or R↓I, where the arrow represents the cleavage point) when the zymogen is activated. To identify the protease domain an RI should be identified, and then the following amino acids compared to the above noted motif.

**[0023]** The protease domains generated herein, however, do not result from activation, which produces a two chain activated product, but rather are single chain polypeptides with the N-terminus include the consensus sequence ↓VVGG, ↓IVGG, ↓VGLL, ↓ILGG or ↓IVNG or other such motif at the N-terminus. As shown herein, such polypeptides, although not the result of activation and not double-chain forms, exhibit proteolytic (catalytic) activity. These protease domain polypeptides are used in assays to screen for agents that modulate the activity of the MTSP. Such assays are also provided herein. In exemplary assays, the affects of test compounds in the ability of a protease domains to proteolytically cleave a known substrate, typically a fluorescently, chromogenically or otherwise detectably labeled substrate, are assessed. Agents, generally compounds, particularly small molecules, that modulate the activity of the protease domain are candidate compounds for modulating the activity of the MTSP. The protease domains can also be used to produce single-chain protease-specific antibodies. The protease domains provided herein include, but are not limited to, the single chain region having an N-terminus at the cleavage site for activation of the zymogen, through the C-terminus, or C-terminal truncated portions thereof that exhibit proteolytic activity as a single-chain polypeptide in *in vitro* proteolysis assays, of any MTSP family member, preferably from a mammal, including and most preferably human, that, for example, is expressed in tumor cells at different levels from non-tumor cells, and that is not expressed on an endothelial cell. These include, but are not limited to: MTSP1 (or matriptase), MTSP3, MTSP4 and MTSP6. Other MTSP protease domains of interest herein, particularly for use in *in vitro* drug screening proteolytic assays, include, but are not limited to: corin (accession nos. AF133845 and AB013874; see,

Yan et al. (1999) *J. Biol. Chem.* 274:14926-14938; Tomia et al. (1998) *J. Biochem.* 124:784-789; Uan et al. (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97:8525-8529; SEQ ID Nos. 61 and 62 for the human protein); enterpeptidase (also designated enterokinase; accession no. U09860 for the human protein; see, Kitamoto et al. (1995) *Biochem.* 27: 4562-4568; Yahagi et al. (1996) *Biochem. Biophys. Res. Commun.* 219:806-812; Kitamoto et al. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91:7588-7592; Matsushima et al. (1994) *J. Biol. Chem.* 269:19976-19982; see SEQ ID Nos. 63 and 64 for the human protein); human airway trypsin-like protease (HAI; accession no. AB002134; see Yamaoka et al. *J. Biol. Chem.* 273:11894-11901; SEQ ID Nos. 65 and 66 for the human protein); hepsin (see, accession nos. M18930, AF030065, X70900; Leytus et al. (1988) *Biochem.* 27: 11895-11901; Vu et al. (1997) *J. Biol. Chem.* 272:31315-31320; and Farley et al. (1993) *Biochem. Biophys. Acta* 1173:350-352; SEQ ID Nos. 67 and 68 for the human protein); TMPRS2 (see, Accession Nos. U75329 and AF113596; Paoloni-Giacobino et al. (1997) *Genomics* 44:309-320; and Jacquinet et al. (2000) *FEBS Lett.* 468: 93-100; SEQ ID Nos. 69 and 70 for the human protein) TMPRSS4 (see, Accession No. NM 016425; Wallrapp et al. (2000) *Cancer* 60:2602-2606; SEQ ID Nos. 71 and 72 for the human protein); and TADG-12 (also designated MTSP6, see SEQ ID Nos. 11 and 12; see International PCT application No. WO 00/52044, which claims priority to U.S. application Ser. No. 09/261,416).

**[0024]** Also provided are muteins of the single chain protease domains and MTSPs, particularly muteins in which the Cys residue in the protease domain that is free (i.e., does not form disulfide linkages with any other Cys residue in the protein) is substituted with another amino acid substitution, preferably with a conservative amino acid substitution or a substitution that does not eliminate the activity, and muteins in which a glycosylation site(s) is eliminated. Muteins in which other conservative amino acid substitutions in which catalytic activity is retained are also contemplated (see, e.g., Table 1, for exemplary amino acid substitutions). See, also, **FIG. 4**, which identifies the free Cys residues in MTSP3, MTSP4 and MTSP6.

**[0025]** Hence, provided herein are members of a family of transmembrane serine protease (MTSP) proteins, and functional domains, especially protease (or catalytic) domains thereof, muteins and other derivatives and analogs thereof. Also provided herein are nucleic acids encoding the MTSPs.

**[0026]** Exemplary MTSPs (see, e.g., SEQ ID No. 1-12, 49 and 50) are provided herein, as are the single chain protease domains thereof as follows: SEQ ID Nos. 1, 2, 49 and 50 set forth amino acid and nucleic acid sequences of MTSP1 and the protease domain thereof; SEQ ID No. 3 sets forth the MTSP3 nucleic acid sequence and SEQ ID No. 4 the encoded MTSP3 amino acids; SEQ ID No. 5 MTSP4 a nucleic acid sequence of the protease domain and SEQ ID No. 6 the encoded MTSP4 amino acid protease domain; SEQ ID No. 7 MTSP4-L a nucleic acid sequence and SEQ ID No. 8 the encoded MTSP4-L amino acid sequence; SEQ ID No. 9 an MTSP4-S encoding nucleic acid sequence and SEQ ID No. 10 the encoded MTSP4-S amino acid sequence; and SEQ ID No. 11 an MTSP6 encoding nucleic acid sequence and SEQ ID No. 12 the encoded MTSP6 amino acid sequence. The single chain protease domains of each are delineated below.

[0027] Nucleic acid molecules that encode a single-chain protease domain or catalytically active portion thereof are provided. Also provided are nucleic acid molecules that hybridize to such MTSP encoding nucleic acid along their full length and encode the protease domain or portion thereof are provided. Hybridization is preferably effected under conditions of at least low, generally at least moderate, and often high stringency.

[0028] Additionally provided herein are antibodies that specifically bind to the MTSPs, cells, combinations, kits and articles of manufacture that contain the nucleic acid encoding the MTSP and/or the MTSP. Further provided herein are prognostic, diagnostic, therapeutic screening methods using MTSPs and the nucleic acids encoding MTSP. Also provided are transgenic non-human animals bearing inactivated genes encoding the MTSP and bearing the genes encoding the MTSP under non-native promotor control are provided. Such animals are useful in animal models of tumor initiation, growth and/or progression models.

[0029] Provided herein are members of a family of membrane serine proteases (MTSP) that are expressed in certain tumor or cancer cells such lung, prostate, colon and breast cancers. In particular, it is shown herein, that MTSPs, particularly, MTSP3, MTSP4 and MTSP6 are expressed in lung carcinoma, breast carcinoma, colon adenocarcinoma and/or ovarian carcinomas as well as in certain normal cells and tissues (see e.g., EXAMPLES for tissue-specific expression profiles of each protein exemplified herein). The MTSPs that are of particular interest herein, are those that are expressed in tumor cells, for example, those that appear to be expressed at different levels in tumor cells from normal cells, or whose functional activity is different in tumor cells from normal cells, such as by an alteration in a substrate therefor, or a cofactor. Hence the MTSP provided herein can serve as diagnostic markers for certain tumors. The level of activated MTSP3, MTSP4 and MTSP6 can be diagnostic of prostate cancer. In addition, MTSP4 is expressed and/or activated in lymphomas, leukemias, lung cancer, breast, prostate and colon cancers. MTSP6 is activated and/or expressed in breast, lung, prostate, colon and ovarian cancers. Furthermore, compounds that modulate the activity of these MTSPs, as assessed by the assays provided herein, particularly the *in vitro* proteolytic assays that use the single chain protease domains, are potential therapeutic candidates for treatment of various malignancies and neoplastic disease.

[0030] Also provided herein are methods of modulating the activity of the MTSPs and screening for compounds that modulate, including inhibit, antagonize, agonize or otherwise alter the activity of the MTSPs. Of particular interest is the extracellular domain of these MTSPs that includes the proteolytic (catalytic) portion of the protein.

[0031] MTSP proteins, including, but not limited to, MTSP3, MTSP4, and MTSP6, including splice variants thereof, and nucleic acids encoding MTSPs, and domains, derivatives and analogs thereof are provided herein. Single chain protease domains, in the N-terminal is that which would be generated by activation of the zymogen, from any MTSP, particularly those that are not expressed in endothelial cells and that are expressed in tumor cells are also provided.

[0032] Antibodies that specifically bind to the MTSP, particularly the single chain protease domain, and any and

all forms of MTSP3 and MTSP4, and cells, combinations, kits and articles of manufacture containing the MTSP proteins, domains thereof, or encoding nucleic acids are also provided herein. Transgenic non-human animals bearing inactivated genes encoding the MTSP and bearing the genes encoding the MTSP under a non-native promotor control are additionally provided herein. Also provided are nucleic acid molecules encoding each of the MTSPs and domains thereof.

[0033] Also provided are plasmids containing any of the nucleic acid molecules provided herein. Cells containing the plasmids are also provided. Such cells include, but are not limited to, bacterial cells, yeast cells, fungal cells, plant cells, insect cells and animal cells.

[0034] Also provided is a method of producing a MTSP by growing the above-described cells under conditions whereby the MTSP is expressed by the cells, and recovering the expressed MTSP protein. Methods for isolating nucleic acid encoding other MTSPs are also provided.

[0035] Also provided are cells, preferably eukaryotic cells, such as mammalian cells and yeast cells, in which the MTSP protein, preferably MTSP3 and MTSP4, is expressed in the surface of the cells. Such cells are used in drug screening assays to identify compounds that modulate the activity of the MTSP protein. These assays including *in vitro* binding assays, and transcription based assays in which signal transduction mediated by the MTSP is assessed.

[0036] Further provided herein are prognostic, diagnostic and therapeutic screening methods using the MTSP and the nucleic acids encoding MTSP. In particular, the prognostic, diagnostic and therapeutic screening methods are used for preventing, treating, or for finding agents useful in preventing or treating, tumors or cancers such as lung carcinoma, colon adenocarcinoma and ovarian carcinoma.

[0037] Also provided are methods for screening for compounds that modulate the activity of any MTSP. The compounds are identified by contacting them with the MTSP and a substrate for the MTSP. A change in the amount of substrate cleaved in the presence of the compounds compared to that in the absence of the compound indicates that the compound modulates the activity of the MTSP. Such compounds are selected for further analyses or for use to modulate the activity of the MTSP, such as inhibitors or agonists. The compounds can also be identified by contacting the substrates with a cell that expresses the MTSP or the extracellular domain or proteolytically active portion thereof. For assays in which the extracellular domain or a proteolytically active portion thereof is employed, the MTSP is any MTSP that is expressed on cells, other than endothelial cells, including, but not limited to MTSP1, MTSP3, MTSP4 and MTSP6.

[0038] Also provided herein are modulators of the activity of the MTSP, especially the modulators obtained according to the screening methods provide herein. Such modulators may have use in treating cancerous conditions, and other neoplastic conditions.

[0039] Pharmaceutical composition containing the protease domains of an MTSP protein, and the MTSP proteins, MTSP3, MTSP4 and MTSP6 are provided herein in a pharmaceutically acceptable carrier or excipient are provided herein.

[0040] Also provided are articles of manufacture that contain the MTSP proteins and protease domains of MTSPs in single chain form. The articles contain a) packaging material; b) the polypeptide (or encoding nucleic acid), particularly the single chain protease domain thereof; and c) a label indicating that the article is for using ins assays for identifying modulators of the activities of an MTSP protein is provided herein.

[0041] Conjugates containing a) a MTSP protease domain in single chain form; and b) a targeting agent linked to the MTSP directly or via a linker, wherein the agent facilitates: i) affinity isolation or purification of the conjugate; ii) attachment of the conjugate to a surface; iii) detection of the conjugate; or iv) targeted delivery to a selected tissue or cell, is provided herein. The conjugate can contain a plurality of agents linked thereto. The conjugate can be a chemical conjugate; and it can be a fusion protein.

[0042] In yet another embodiment, the targeting agent is a protein or peptide fragment. The protein or peptide fragment can include a protein binding sequence, a nucleic acid binding sequence, a lipid binding sequence, a polysaccharide binding sequence, or a metal binding sequence.

[0043] Method of diagnosing a disease or disorder characterized by detecting an aberrant level of an MTSP, particularly an MTSP3, MTSP4 or MTSP 6, in a subject is provided. The method can be practiced by measuring the level of the DNA, RNA, protein or functional activity of the MTSP. An increase or decrease in the level of the DNA, RNA, protein or functional activity of the MTSP, relative to the level of the DNA, RNA, protein or functional activity found in an analogous sample not having the disease or disorder (or other suitable control) is indicative of the presence of the disease or disorder in the subject or other relative any other suitable control.

[0044] Combinations are provided herein. The combination can include: a) an inhibitor of the activity of an MTSP; and b) an anti-cancer treatment or agent. The MTSP inhibitor and the anti-cancer agent can be formulated in a single pharmaceutical composition or each is formulated in a separate pharmaceutical composition. The MTSP inhibitor can be an antibody or a fragment or binding portion thereof against the MTSP, such as an antibody that specifically binds to the protease domain, an inhibitor of the MTSP production, or an inhibitor of the MTSP membrane-localization or an inhibitor of MTSP activation. Other MTSP inhibitors include, but are not limited to, an antisense nucleic acid encoding the MTSP, particularly a portion of the protease domain; a nucleic acid encoding at least a portion of a gene encoding the MTSP with a heterologous nucleotide sequence inserted therein such that the heterologous sequence inactivates the biological activity encoded MTSP or the gene encoding it. The portion of the gene encoding the MTSP preferably flanks the heterologous sequence to promote homologous recombination with a genomic gene encoding the MTSP.

[0045] Also, provided are methods for treating or preventing a tumor or cancer in a mammal by administering to a mammal an effective amount of an inhibitor of an MTSP3, MTSP4 or MTSP6, whereby the tumor or cancer is treated or prevented. The MTSP inhibitor used in the treatment or for prophylaxis is administered with a pharmaceutically acceptable carrier or excipient. The mammal treated can be

a human. The treatment or prevention method can additionally include administering an anti-cancer treatment or agent simultaneously with or subsequently or before administration of the MTSP inhibitor.

[0046] Also provided is a recombinant non-human animal in which an endogenous gene of an MTSP has been deleted or inactivated by homologous recombination or insertional mutagenesis of the animal or an ancestor thereof. A recombinant non-human animal is provided herein, where the gene of an MTSP is under control of a promoter that is not the native promoter of the gene or that is not the native promoter of the gene in the non-human animal or where the nucleic acid encoding the MTSP is heterologous to the non-human animal and the promoter is the native or a non-native promoter.

[0047] Also provided are methods of treatments of tumors by administering a prodrug that is activated by an MTSP that is expressed or active in tumor cells, particularly those in which its functional activity in tumor cells is greater than in non-tumor cells. The prodrug is administered and, upon administration, active MTSP expressed on cells cleaves the prodrug and releases active drug in the vicinity of these cells. The active anti-cancer drug accumulates in the vicinity of the tumor. This is particularly useful in instances in which an MTSP is expressed or active in greater quantity, higher level or predominantly in tumor cells compared to other cells.

#### BRIEF DESCRIPTION OF DRAWINGS

[0048] FIG. 1 illustrates the domain organization of the MTSP3;

[0049] FIG. 2 illustrates the domain organization of the MTSP4 splice variants and domains thereof; MTSP4-L includes a transmembrane domain, a CUB domain, a low density lipoprotein receptor (LDLR) domains, and a serine protease catalytic domain; MTSP4-S lacking the portion between amino acids 136-279.

[0050] FIG. 3 depicts the domain organization of MTSP6.

[0051] FIG. 4 provides an alignment of the C-terminal portions of MTSP3, the two splice variant-encoded forms of MTSP4, and MTSP6, that encompasses the protease domains thereof; the figure shows the cleavage sites, which form the N-terminus of the protease domain of each protein; a potential glycosylation site is noted and the free Cys residues in the protease domain of each are noted (\*). Muteins of each protein may be prepared by replacing the residues in the glycosylation site, particularly the N residue, and the free Cys residues, with preferably conservative amino acid residues. Such muteins are also provided herein.

#### DETAILED DESCRIPTION OF THE INVENTION

[0052] A. Definitions

[0053] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other publications and sequences from GenBank and other data bases referred to herein are incorporated by reference in their entirety.

[0054] As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, (1972) *Biochem.* 11:942-944).

[0055] As used herein, serine protease refers to a diverse family of proteases wherein a serine residue is involved in the hydrolysis of proteins or peptides. The serine residue can be part of the catalytic triad mechanism, which includes a serine, a histidine and an aspartic acid in the catalysis, or be part of the hydroxyl/ $\epsilon$ -amine or hydroxyl/ $\alpha$ -amine catalytic dyad mechanism, which involves a serine and a lysine in the catalysis.

[0056] As used herein, "transmembrane serine protease (MTSP)" refers to a family of transmembrane serine proteases that share common structural features as described herein (see, also Hooper et al. (2001) *J. Biol. Chem.* 276:857-860). Thus, reference, for example, to "MTSP" encompasses all proteins encoded by the MTSP gene family, including but are not limited to: MTSP1, MTSP3, MTSP4 and MTSP6, or an equivalent molecule obtained from any other source or that has been prepared synthetically or that exhibits the same activity. Other MTSPs include, but are not limited to, corin, enterpeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4. Sequences of encoding nucleic molecules and the encoded amino acid sequences of exemplary MTSPs and/or domains thereof are set forth in SEQ ID Nos. 1-12, 49, 50 and 61-72. The term also encompass MTSPs with conservative amino acid substitutions that do not substantially alter activity of each member, and also encompasses splice variants thereof. Suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Of particular interest are MTSPs of mammalian, including human, origin. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson et al. *Molecular Biology of the Gene*, 4th Edition, 1987, The Bejaamin/Cummings Pub. co., p. 224).

[0057] As used herein, a "protease domain of an MTSP" refers to the protease domain of MTSP that is located within the extracellular domain of a MTSP and exhibits serine proteolytic activity. It includes at least the smallest fragment thereof that acts catalytically as a single chain form. Hence it is at least the minimal portion of the extracellular domain that exhibits proteolytic activity as assessed by standard assays in vitro assays. Those of skill in this art recognize that such protease domain is the portion of the protease that is structurally equivalent to the trypsin or chymotrypsin fold.

[0058] Exemplary MTSP proteins, with the protease domains indicated, are illustrated in FIGS. 1-3. Smaller portions thereof that retain protease activity are contemplated. The protease domains vary in size and constitution, including insertions and deletions in surface loops. They retain conserved structure, including at least one of the active site triad, primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a portion of a MTSP, as defined herein, and is homologous to

a domain of other MTSPs, such as corin, enterpeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4, which have been previously identified; it was not recognized, however, that an isolated single chain form of the protease domain could function proteolytically in in vitro assays. As with the larger class of enzymes of the chymotrypsin (S1) fold (see, e.g., Internet accessible MEROPS data base), the MTSPs protease domains share a high degree of amino acid sequence identity. The His, Asp and Ser residues necessary for activity are present in conserved motifs. The activation site, which results in the N-terminus of second chain in the two chain forms is has a conserved motif and readily can be identified (see, e.g., amino acids 801-806, SEQ ID No. 62, amino acids 406-410, SEQ ID No. 64; amino acids 186-190, SEQ ID No. 66; amino acids 161-166, SEQ ID No. 68; amino acids 255-259, SEQ ID No. 70; amino acids 190-194, SEQ ID No. 72).

[0059] As used herein, the catalytically active domain of an MTSP refers to the protease domain. Reference to the protease domain of an MTSP refers includes the single and double-chain forms of any of these proteins. The zymogen form of each protein is single chain form, which can be converted to the active two chain form by cleavage. The protease domain may also be converted to a two chain form. By active form is meant a form active in vivo.

[0060] Significantly, it is shown herein, that, at least in vitro, the single chain forms of the MTSPs and the catalytic domains or proteolytically active portions thereof (typically C-terminal truncations) thereof exhibit protease activity. Hence provided herein are isolated single chain forms of the protease domains of MTSPs and their use in in vitro drug screening assays for identification of agents that modulate the activity thereof.

[0061] As used herein an MTSP3, whenever referenced herein, includes at least one or all of or any combination of:

[0062] a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 3;

[0063] a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of low, moderate or high stringency to the sequence of nucleotides set forth in SEQ ID No. 3;

[0064] a polypeptide that comprises the sequence of amino acids set forth as amino acids 205-437 of SEQ ID No. 4;

[0065] a polypeptide that comprises a sequence of amino acids having at least about 85% or 90% sequence identity with the sequence of amino acids set forth in SEQ ID No. 4; and/or

[0066] a splice variant of the MTSP3 set forth in SEQ ID Nos. 3 and 4.

[0067] The MTSP3 may be from any animal, particularly a mammal, and includes but are not limited to, humans, rodents, fowl, ruminants and other animals. The full length zymogen or double chain activated form is contemplated or any domain thereof, including the protease domain, which can be a double chain activated form, or a single chain form.

[0068] As used herein an MTSP4, whenever referenced herein, includes at least one or all of or any combination of:

- [0069] a polypeptide encoded by the sequence of nucleotides set forth in any of SEQ ID No. 5, 7 or 9;
- [0070] a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of low, moderate or high stringency to the sequence of nucleotides set forth in any of SEQ ID Nos. 5, 7 or 9;
- [0071] a polypeptide that comprises the sequence of amino acids set forth in any of SEQ ID Nos. 6, 8 or 10;
- [0072] a polypeptide that comprises a sequence of amino acids having at least about 85% or 90% or 95% sequence identity with the sequence of amino acids set forth in SEQ ID No. 6, 8 or 10; and/or
- [0073] a splice variant of the MTSP4s set forth in SEQ ID Nos. 7-10.
- [0074] The MTSP4 may be from any animal, particularly a mammal, and includes but are not limited to, humans, rodents, fowl, ruminants and other animals. The full length zymogen or double chain activated form is contemplated or any domain thereof, including the protease domain, which can be a double chain activated form, or a single chain form.
- [0075] As used herein an MTSP6, whenever referenced herein, includes at least one or all of or any combination of:
- [0076] a polypeptide encoded by the sequence of nucleotides set forth in any of SEQ ID No. 11;
- [0077] a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of low, moderate or high stringency to the sequence of nucleotides set forth in any of SEQ ID Nos. 11;
- [0078] a polypeptide that comprises the sequence of amino acids set forth in any of SEQ ID Nos. 12;
- [0079] a polypeptide that comprises a sequence of amino acids having at least about 90% or 95% or 98% sequence identity with the sequence of amino acids set forth in SEQ ID No. 12; and/or
- [0080] a splice variant of the MTSP4s set forth in SEQ ID No. 12.
- [0081] The MTSP6 may be from any animal, particularly a mammal, and includes but are not limited to, humans, rodents, fowl, ruminants and other animals. The full length zymogen or double chain activated form is contemplated or any domain thereof, including the protease domain, which can be a double chain activated form, or a single chain form. Of particular interest herein is the MTSP6 of SEQ ID No. 12.
- [0082] As used herein, a human protein is one encoded by DNA present in the genome of a human, including all allelic variants and conservative variations as long as they are not variants found in other mammals.
- [0083] As used herein, a "nucleic acid encoding a protease domain or catalytically active portion of a MTSP" shall be construed as referring to a nucleic acid encoding only the recited single chain protease domain or active portion thereof, and not the other contiguous portions of the MTSP as a continuous sequence.
- [0084] As used herein, a CUB domain is a motif that mediates protein-protein interactions in complement components C1r/C1s and has also been identified in various proteins involved in developmental processes.
- [0085] As used herein, catalytic activity refers to the activity of the MTSP as a serine proteases. Function of the MTSP refers to its function in tumor biology, including promotion of or involvement in tumorigenesis, metastasis or carcinogenesis, and also roles in signal transduction.
- [0086] As used herein, a "propeptide" or "pro sequence" is sequence of amino acids positioned at the amino terminus of a mature biologically active polypeptide. When so-positioned, the resulting polypeptide is called a zymogen. Zymogens, generally, are biologically inactive and can be converted to mature active polypeptides by catalytic or autocatalytic cleavage of the propeptide from the zymogen. A zymogen is an enzymatically inactive protein that is converted to a proteolytic enzyme by the action of an activator. Cleavage may be effected autocatalytically.
- [0087] As used herein, "disease or disorder" refers to a pathological condition in an organism resulting from, e.g., infection or genetic defect, and characterized by identifiable symptoms.
- [0088] As used herein, neoplasm (neoplasia) refers to abnormal new growth, and thus means the same as tumor, which may be benign or malignant. Unlike hyperplasia, neoplastic proliferation persists even in the absence of the original stimulus.
- [0089] As used herein, neoplastic disease refers to any disorder involving cancer, including tumor development, growth, metastasis and progression.
- [0090] As used herein, cancer refers to a general term for diseases caused by any type of malignant tumor.
- [0091] As used herein, malignant, as applies to tumors, refers to primary tumors that have the capacity of metastasis with loss of growth control and positional control.
- [0092] As used herein, an anti-cancer agent (used interchangeably with "anti-tumor or anti-neoplastic agent") refers to any agents used in the anti-cancer treatment. These include any agents, when used alone or in combination with other compounds, that can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with neoplastic disease, tumor and cancer, and can be used in methods, combinations and compositions provided herein. Non-limiting examples of anti-neoplastic agents include anti-angiogenic agents, alkylating agents, antimetabolite, certain natural products, platinum coordination complexes, anthracenediones, substituted ureas, methylhydrazine derivatives, adrenocortical suppressants, certain hormones, antagonists and anti-cancer polysaccharides.
- [0093] As used herein, a splice variant refers to a variant produced by differential processing of a primary transcript of genomic DNA that results in more than one type of mRNA. Splice variants of MTSPs are provided herein.
- [0094] As used herein, angiogenesis is intended to broadly encompass the totality of processes directly or indirectly involved in the establishment and maintenance of new vasculature (neovascularization), including, but not limited to, neovascularization associated with tumors.

**[0095]** As used herein, anti-angiogenic treatment or agent refers to any therapeutic regimen and compound, when used alone or in combination with other treatment or compounds, that can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with undesired and/or uncontrolled angiogenesis. Thus, for purposes herein an anti-angiogenic agent refers to an agent that inhibits the establishment or maintenance of vasculature. Such agents include, but are not limited to, anti-tumor agents, and agents for treatments of other disorders associated with undesirable angiogenesis, such as diabetic retinopathies, restenosis, hyperproliferative disorders and others.

**[0096]** As used herein, non-anti-angiogenic anti-tumor agents refer to anti-tumor agents that do not act primarily by inhibiting angiogenesis. As used herein, pro-angiogenic agents are agents that promote the establishment or maintenance of the vasculature. Such agents include agents for treating cardiovascular disorders, including heart attacks and strokes.

**[0097]** As used herein, undesired and/or uncontrolled angiogenesis refers to pathological angiogenesis wherein the influence of angiogenesis stimulators outweighs the influence of angiogenesis inhibitors. As used herein, deficient angiogenesis refers to pathological angiogenesis associated with disorders where there is a defect in normal angiogenesis resulting in aberrant angiogenesis or an absence or substantial reduction in angiogenesis.

**[0098]** As used herein, endotheliase refers to a mammalian protein, including humans, that has a transmembrane domain and is expressed on the surface of endothelial cells and includes a protease domain, particularly an extracellular protease domain, and is preferably a serine protease. Thus, reference, for example, to endotheliase encompasses all proteins encoded by the endotheliase gene family, or an equivalent molecule obtained from any other source or that has been prepared synthetically or that exhibits the same activity. The endotheliase gene family are transmembrane proteases expressed in endothelial cells. Endotheliases are excluded from the MTSPs contemplated herein.

**[0099]** As used herein, the protease domain of an endotheliase refers to the polypeptide portion of the endotheliase that is the extracellular portion that exhibits protease activity. The protease domain is a polypeptide that includes at least the minimum number of amino acids, generally more than 50 or 100, required for protease activity. Protease activity may be assessed empirically, such as by testing the polypeptide for its ability to act as a protease. Assays, such as in the assays described in the EXAMPLES, except employing a known substrate in place of the test compounds as described in the Examples may be used. Furthermore, since proteases, particularly serine proteases, have characteristic structures and sequences or motifs, the protease domain may be readily identified by such structure and sequence or motif.

**[0100]** As used herein, the protease domain of an MTSP protein refers to the protease domain of an MTSP that is located within or is the extracellular domain of an MTSP and exhibits serine proteolytic activity. Hence it is at least the minimal portion of the extracellular domain that exhibits proteolytic activity as assessed by standard assays in vitro. It refers, herein, to a single chain form heretofore thought to

be inactive. Exemplary protease domains include at least a sufficient portion of sequences of amino acids set forth as amino acids 615-855 in SEQ ID No. 2 (encoded by nucleotides 1865-2587 in SEQ ID No. 1; see also SEQ ID Nos. 49 and 50) from MTSP1, amino acids 205-437 of SEQ ID NO. 4 from MTSP3, SEQ ID No. 6, which sets forth the protease domain of MTSP4, and amino acids 217-443 of SEQ ID No. 11 from MTSP6. Also contemplated are nucleic acid molecules that encode polypeptide that has proteolytic activity in an in vitro proteolysis assay and that have at least 80%, 85%, 90% or 95% sequence identity with the full length of a protease domain of an MTSP protein, or that hybridize along their full length to a nucleic acids that encode a protease domain, particularly under conditions of moderate, generally high, stringency.

**[0101]** For each of these protease domains, residues at the N-terminus can be critical for activity, since it has been shown that an Asp in the N-terminus of such proteases is essential for formation of the catalytically active conformation upon activation cleavage of the zymogen form of the protease. It is shown herein that the protease domain of the single chain form of the protease is catalytically active. Hence the protease domain will require the N-terminal amino acids; the c-terminus portion may be truncated. The amount that can be removed can be determined empirically by testing the protein for protease activity in an in vitro assays that assesses catalytic cleavage.

**[0102]** Hence smaller portions of the protease domains, particularly the single chain domains, thereof that retain protease activity are contemplated. Such smaller versions will generally be C-terminal truncated versions of the protease domains. The protease domains vary in size and constitution, including insertions and deletions in surface loops. Such domains exhibit conserved structure, including at least one structural feature, such as the active site triad, primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a single chain portion of an MTSP, as defined herein, but is homologous in its structural features and retention of sequence of similarity or homology the protease domain of chymotrypsin or trypsin. Most significantly, the polypeptide will exhibit proteolytic activity as a single chain.

**[0103]** As used herein, by homologous means about greater than 25% nucleic acid sequence identity, preferably 25% 40%, 60%, 80%, 90% or 95%. The terms "homology" and "identity" are often used interchangeably. In general, sequences are aligned so that the highest order match is obtained (see, e.g.: *Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carillo et al. (1988) *SIAM J Applied Math* 48:1073).

**[0104]** By sequence identity, the number of conserved amino acids are determined by standard alignment algorithms programs, and are used with default gap penalties established by each supplier. Substantially homologous

nucleic acid molecules would hybridize typically at moderate stringency or at high stringency all along the length of the nucleic acid of interest. Also contemplated are nucleic acid molecules that contain degenerate codons in place of codons in the hybridizing nucleic acid molecule.

**[0105]** Whether any two nucleic acid molecules have nucleotide sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" can be determined using known computer algorithms such as the "FASTA" program, using for example, the default parameters as in Pearson et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:2444 (other programs include the GCG program package (Devereux, J., et al, *Nucleic Acids Research* 12(1):387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S. F., et al., *J Molec Biol* 215:403 (1990); Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo et al. (1988) *SIAM J Applied Math* 48:1073). For example, the BLAST function of the National Center for Biotechnology Information database may be used to determine identity. Other commercially or publicly available programs include, DNASTar "MegAlign" program (Madison, Wis.) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison Wis.). Percent homology or identity of proteins and/or nucleic acid molecules may be determined, for example, by comparing sequence information using a GAP computer program (e.g., Needleman et al. (1970) *J. Mol. Biol.* 48:443, as revised by Smith and Waterman ((1981) *Adv. Appl. Math.* 2:482). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. Default parameters for the GAP program may include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov et al. (1986) *Nucl. Acids Res.* 14:6745, as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

**[0106]** Therefore, as used herein, the term "identity" represents a comparison between a test and a reference polypeptide or polynucleotide. For example, a test polypeptide may be defined as any polypeptide that is 90% or more identical to a reference polypeptide. As used herein, the term at least "90% identical to" refers to percent identities from 90 to 99.99 relative to the reference polypeptides. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polynucleotide length of 100 amino acids are compared. No more than 10% (i.e., 10 out of 100) amino acids in the test polypeptide differs from that of the reference polypeptides. Similar comparisons may be made between a test and reference polynucleotides. Such differences may be represented as point mutations randomly distributed over the entire length of an amino acid sequence or they may be clustered in one or more locations of varying length up to the maximum allowable, e.g. 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, or deletions. At level of homologies or identities above about 85-90%, the result should be independent of the program and gap param-

eters set; such high levels of identity readily can be assessed, often without relying on software.

**[0107]** As used herein, primer refers to an oligonucleotide containing two or more deoxyribonucleotides or ribonucleotides, preferably more than three, from which synthesis of a primer extension product can be initiated. Experimental conditions conducive to synthesis include the presence of nucleoside triphosphates and an agent for polymerization and extension, such as DNA polymerase, and a suitable buffer, temperature and pH.

**[0108]** As used herein, animals include any animal, such as, but are not limited to, goats, cows, deer, sheep, rodents, pigs and humans. Non-human animals, exclude humans as the contemplated animal. The MTSPs provided herein are from any source, animal, plant, prokaryotic and fungal. Preferred MTSPs are of animal origin, preferably mammalian origin.

**[0109]** As used herein, genetic therapy involves the transfer of heterologous DNA to the certain cells, target cells, of a mammal, particularly a human, with a disorder or conditions for which such therapy is sought. The DNA is introduced into the selected target cells in a manner such that the heterologous DNA is expressed and a therapeutic product encoded thereby is produced. Alternatively, the heterologous DNA may in some manner mediate expression of DNA that encodes the therapeutic product, or it may encode a product, such as a peptide or RNA that in some manner mediates, directly or indirectly, expression of a therapeutic product. Genetic therapy may also be used to deliver nucleic acid encoding a gene product that replaces a defective gene or supplements a gene product produced by the mammal or the cell in which it is introduced. The introduced nucleic acid may encode a therapeutic compound, such as a growth factor inhibitor thereof, or a tumor necrosis factor or inhibitor thereof, such as a receptor thereof, that is not normally produced in the mammalian host or that is not produced in therapeutically effective amounts or at a therapeutically useful time. The heterologous DNA encoding the therapeutic product may be modified prior to introduction into the cells of the afflicted host in order to enhance or otherwise alter the product or expression thereof. Genetic therapy may also involve delivery of an inhibitor or repressor or other modulator of gene expression.

**[0110]** As used herein, heterologous DNA is DNA that encodes RNA and proteins that are not normally produced in vivo by the cell in which it is expressed or that mediates or encodes mediators that alter expression of endogenous DNA by affecting transcription, translation, or other regulatable biochemical processes. Heterologous DNA may also be referred to as foreign DNA. Any DNA that one of skill in the art would recognize or consider as heterologous or foreign to the cell in which is expressed is herein encompassed by heterologous DNA. Examples of heterologous DNA include, but are not limited to, DNA that encodes traceable marker proteins, such as a protein that confers drug resistance, DNA that encodes therapeutically effective substances, such as anti-cancer agents, enzymes and hormones, and DNA that encodes other types of proteins, such as antibodies. Antibodies that are encoded by heterologous DNA may be secreted or expressed on the surface of the cell in which the heterologous DNA has been introduced.

**[0111]** Hence, herein heterologous DNA or foreign DNA, includes a DNA molecule not present in the exact orientation

and position as the counterpart DNA molecule found in the genome. It may also refer to a DNA molecule from another organism or species (i.e., exogenous).

**[0112]** As used herein, a therapeutically effective product is a product that is encoded by heterologous nucleic acid, typically DNA, that, upon introduction of the nucleic acid into a host, a product is expressed that ameliorates or eliminates the symptoms, manifestations of an inherited or acquired disease or that cures the disease.

**[0113]** As used herein, recitation that a polypeptide consists essentially of the protease domain means that the only MTSP portion of the polypeptide is a protease domain or a catalytically active portion thereof. The polypeptide may optionally, and generally will, include additional non-MTSP-derived sequences of amino acids.

**[0114]** As used herein, cancer or tumor treatment or agent refers to any therapeutic regimen and/or compound that, when used alone or in combination with other treatments or compounds, can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with deficient angiogenesis.

**[0115]** As used herein, domain refers to a portion of a molecule, e.g., proteins or nucleic acids, that is structurally and/or functionally distinct from other portions of the molecule.

**[0116]** As used herein, protease refers to an enzyme catalyzing hydrolysis of proteins or peptides. For purposes herein, the protease domain is a single chain form of an MTSP protein. For MTSP3 and MTSP4 the protease domain also includes two chain forms.

**[0117]** As used herein, catalytic activity refers to the activity of the MTSP as a protease as assessed in in vitro proteolytic assays that detect proteolysis of a selected substrate.

**[0118]** As used herein, nucleic acids include DNA, RNA and analogs thereof, including protein nucleic acids (PNA) and mixture thereof. Nucleic acids can be single or double stranded. When referring to probes or primers, optionally labeled, with a detectable label, such as a fluorescent or radiolabel, single-stranded molecules are contemplated. Such molecules are typically of a length such that they are statistically unique of low copy number (typically less than 5, preferably less than 3) for probing or priming a library. Generally a probe or primer contains at least 14, 16 or 30 contiguous of sequence complementary to or identical a gene of interest. Probes and primers can be 10, 20, 30, 50, 100 or more nucleic acids long.

**[0119]** As used herein, nucleic acid encoding a fragment or portion of an MTSP refers to a nucleic acid encoding only the recited fragment or portion of MTSP, and not the other contiguous portions of the MTSP.

**[0120]** As used herein, heterologous or foreign DNA and RNA are used interchangeably and refer to DNA or RNA that does not occur naturally as part of the genome in which it is present or which is found in a location or locations in the genome that differ from that in which it occurs in nature. Heterologous nucleic acid is generally not endogenous to the cell into which it is introduced, but has been obtained from another cell or prepared synthetically. Generally, although

not necessarily, such nucleic acid encodes RNA and proteins that are not normally produced by the cell in which it is expressed. Any DNA or RNA that one of skill in the art would recognize or consider as heterologous or foreign to the cell in which it is expressed is herein encompassed by heterologous DNA. Heterologous DNA and RNA may also encode RNA or proteins that mediate or alter expression of endogenous DNA by affecting transcription, translation, or other regulatable biochemical processes.

**[0121]** As used herein, operative linkage of heterologous DNA to regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences refers to the relationship between such DNA and such sequences of nucleotides. For example, operative linkage of heterologous DNA to a promoter refers to the physical relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA in reading frame.

**[0122]** As used herein, a sequence complementary to at least a portion of an RNA, with reference to antisense oligonucleotides, means a sequence having sufficient complementarity to be able to hybridize with the RNA, preferably under moderate or high stringency conditions, forming a stable duplex; in the case of double-stranded MTSP antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize depends on the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a MTSP encoding RNA it can contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

**[0123]** For purposes herein, conservative amino acid substitutions may be made in any of MTSPs and protease domains thereof provided that the resulting protein exhibits protease activity. Conservative amino acid substitutions, such as those set forth in Table 1, are those that do not eliminate proteolytic activity. Suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson et al. *Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. co., p. 224). Also included within the definition, is the catalytically active fragment of an MTSP, particularly a single chain protease portion. Conservative amino acid substitutions are made, for example, in accordance with those set forth in TABLE 1 as follows:

TABLE 1

Original residue	Conservative substitution
Ala (A)	Gly; Ser; Abu
Arg (R)	Lys; orn
Asn (N)	Gln; His
Cys (C)	Ser

TABLE 1-continued

Original residue	Conservative substitution
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala; Pro
His (H)	Asn; Gln
Ile (I)	Leu; Val; Met; Nle; Nva
Leu (L)	Ile; Val; Met; Nle; Nv
Lys (K)	Arg; Gln; Glu
Met (M)	Leu; Tyr; Ile; NLe Val
Ornithine	Lys; Arg
Phe (F)	Met; Leu; Tyr
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu; Met; Nle; Nv

[0124] Other substitutions are also permissible and may be determined empirically or in accord with known conservative substitutions.

[0125] As used herein, Abu is 2-aminobutyric acid; Orn is ornithine.

[0126] As used herein, the amino acids, which occur in the various amino acid sequences appearing herein, are identified according to their well-known, three-letter or one-letter abbreviations. The nucleotides, which occur in the various DNA fragments, are designated with the standard single-letter designations used routinely in the art.

[0127] As used herein, a splice variant refers to a variant produced by differential processing of a primary transcript of genomic DNA that results in more than one type of mRNA.

[0128] As used herein, a probe or primer based on a nucleotide sequence disclosed herein, includes at least 10, 14, preferably at least 16 or 30 or 100 contiguous sequence of nucleotides of SEQ ID Nos. 1, 3, 5, 7, 9 or 11.

[0129] As used herein, amelioration of the symptoms of a particular disorder by administration of a particular pharmaceutical composition refers to any lessening, whether permanent or temporary, lasting or transient that can be attributed to or associated with administration of the composition.

[0130] As used herein, antisense polynucleotides refer to synthetic sequences of nucleotide bases complementary to mRNA or the sense strand of double stranded DNA. Admixture of sense and antisense polynucleotides under appropriate conditions leads to the binding of the two molecules, or hybridization. When these polynucleotides bind to (hybridize with) mRNA, inhibition of protein synthesis (translation) occurs. When these polynucleotides bind to double stranded DNA, inhibition of RNA synthesis (transcription) occurs. The resulting inhibition of translation and/or transcription leads to an inhibition of the synthesis of the protein encoded by the sense strand. Antisense nucleic acid molecule typically contain a sufficient number of nucleotides to specifically bind to a target nucleic acid, generally at least 5 contiguous nucleotides, often at least 14 or 16 or 30 contiguous nucleotides or modified nucleotides complementary to the coding portion of a nucleic acid molecule that encodes

a gene of interest, for example, nucleic acid encoding a single chain protease domain of an MTSP.

[0131] As used herein, an array refers to a collection of elements, such as antibodies, containing three or more members. An addressable array is one in which the members of the array are identifiable, typically by position on a solid phase support. Hence, in general the members of the array will be immobilized to discrete identifiable loci on the surface of a solid phase.

[0132] As used herein, antibody refers to an immunoglobulin, whether natural or partially or wholly synthetically produced, including any derivative thereof that retains the specific binding ability the antibody. Hence antibody includes any protein having a binding domain that is homologous or substantially homologous to an immunoglobulin binding domain. Antibodies include members of any immunoglobulin claims, including IgG, IgM, IgA, IgD and IgE.

[0133] As used herein, antibody fragment refers to any derivative of an antibody that is less than full length, retaining at least a portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab)<sub>2</sub>, single-chain Fvs (scFV), FV, dsFV diabody and Fd fragments. The fragment can include multiple chains linked together, such as by disulfide bridges. An antibody fragment generally contains at least about 50 amino acids and typically at least 200 amino acids.

[0134] As used herein, an Fv antibody fragment is composed of one variable heavy domain (V<sub>H</sub>) and one variable light domain linked by noncovalent interactions.

[0135] As used herein, a dsFV refers to an Fv with an engineered intermolecular disulfide bond, which stabilizes the V<sub>H</sub>-V<sub>L</sub> pair.

[0136] As used herein, an F(ab)<sub>2</sub> fragment is an antibody fragment that results from digestion of an immunoglobulin with pepsin at pH 4.0-4.5; it may be recombinantly produced.

[0137] As used herein, Fab fragments is an antibody fragment that results from digestion of an immunoglobulin with papain; it may be recombinantly produced.

[0138] As used herein, scFVs refer to antibody fragments that contain a variable light chain (V<sub>L</sub>) and variable heavy chain (V<sub>H</sub>) covalently connected by a polypeptide linker in any order. The linker is of a length such that the two variable domains are bridged without substantial interference. Preferred linkers are (Gly-Ser)<sub>n</sub> residues with some Glu or Lys residues dispersed throughout to increase solubility.

[0139] As used herein, humanized antibodies refer to antibodies that are modified to include human sequences of amino acids so that administration to a human will not provoke an immune response. Methods for preparation of such antibodies are known. For example, the hybridoma that expresses the monoclonal antibody is altered by recombinant DNA techniques to express an antibody in which the amino acid composition of the non-variable regions is based on human antibodies. Computer programs have been designed to identify such regions.

[0140] As used herein, diabodies are dimeric scFV; diabodies typically have shorter peptide linkers than scFVs, and they preferentially dimerize.

[0141] As used herein, humanized antibodies refer to antibodies that are modified to include human sequences of amino acids so that administration to a human will not provoke an immune response. Methods for preparation of such antibodies are known. For example, the hybridoma that expresses the monoclonal antibody is altered by recombinant DNA techniques to express an antibody in which the amino acid composition of the non-variable regions is based on human antibodies. Computer programs have been designed to identify such regions.

[0142] As used herein, production by recombinant means by using recombinant DNA methods means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA.

[0143] As used herein the term assessing is intended to include quantitative and qualitative determination in the sense of obtaining an absolute value for the activity of an MTSP, or a domain thereof, present in the sample, and also of obtaining an index, ratio, percentage, visual or other value indicative of the level of the activity. Assessment may be direct or indirect and the chemical species actually detected need not of course be the proteolysis product itself but may for example be a derivative thereof or some further substance.

[0144] As used herein, biological activity refers to the in vivo activities of a compound or physiological responses that result upon in vivo administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical activity of such compounds, compositions and mixtures. Biological activities may be observed in in vitro systems designed to test or use such activities. Thus, for purposes herein the biological activity of a luciferase is its oxygenase activity whereby, upon oxidation of a substrate, light is produced.

[0145] As used herein, a combination refers to any association between two or among more items.

[0146] As used herein, a composition refers to a any mixture. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

[0147] As used herein, a conjugate refers to the compounds provided herein that include one or more MTSPs, particularly single chain protease domains thereof, and one or more targeting agents. These conjugates include those produced by recombinant means as fusion proteins, those produced by chemical means, such as by chemical coupling, through, for example, coupling to sulfhydryl groups, and those produced by any other method whereby at least one MTSP, or a domain thereof, is linked, directly or indirectly via linker(s) to a targeting agent.

[0148] As used herein, a targeting agent, is any moiety, such as a protein or effective portion thereof, that provides specific binding of the conjugate to a cell surface receptor, which, preferably, internalizes the conjugate or MTSP portion thereof. A targeting agent may also be one that promotes or facilitates, for example, affinity isolation or purification of the conjugate; attachment of the conjugate to a surface; or detection of the conjugate or complexes containing the conjugate.

[0149] As used herein, an antibody conjugate refers to a conjugate in which the targeting agent is an antibody.

[0150] As used herein, humanized antibodies refer to antibodies that are modified to include human sequences of amino acids so that administration to a human will not provoke an immune response. Methods for preparation of such antibodies are known. For example, the hybridoma that expresses the monoclonal antibody is altered by recombinant DNA techniques to express an antibody in which the amino acid composition of the non-variable regions is based on human antibodies. Computer programs have been designed to identify such regions.

[0151] As used herein, derivative or analog of a molecule refers to a portion derived from or a modified version of the molecule.

[0152] As used herein, fluid refers to any composition that can flow. Fluids thus encompass compositions that are in the form of semi-solids, pastes, solutions, aqueous mixtures, gels, lotions, creams and other such compositions.

[0153] As used herein, an effective amount of a compound for treating a particular disease is an amount that is sufficient to ameliorate, or in some manner reduce the symptoms associated with the disease. Such amount may be administered as a single dosage or may be administered according to a regimen, whereby it is effective. The amount may cure the disease but, typically, is administered in order to ameliorate the symptoms of the disease. Repeated administration may be required to achieve the desired amelioration of symptoms.

[0154] As used herein equivalent, when referring to two sequences of nucleic acids means that the two sequences in question encode the same sequence of amino acids or equivalent proteins. When equivalent is used in referring to two proteins or peptides, it means that the two proteins or peptides have substantially the same amino acid sequence with only conservative amino acid substitutions (see, e.g., Table 1, above) that do not substantially alter the activity or function of the protein or peptide. When equivalent refers to a property, the property does not need to be present to the same extent [e.g., two peptides can exhibit different rates of the same type of enzymatic activity], but the activities are preferably substantially the same. Complementary, when referring to two nucleotide sequences, means that the two sequences of nucleotides are capable of hybridizing, preferably with less than 25%, more preferably with less than 15%, even more preferably with less than 5%, most preferably with no mismatches between opposed nucleotides. Preferably the two molecules will hybridize under conditions of high stringency.

[0155] As used herein, an agent that modulates the activity of a protein or expression of a gene or nucleic acid either decreases or increases or otherwise alters the activity of the protein or, in some manner up- or down-regulates or otherwise alters expression of the nucleic acid in a cell.

[0156] As used herein, inhibitor of an the activity of an MTSP encompasses any substances that prohibit or decrease production, post-translational modification(s), maturation, or membrane localization of the MTSP or any substances that interfere with or decrease the proteolytic efficacy of thereof, particular of a single chain form in vitro.

[0157] As used herein, a method for treating or preventing neoplastic disease means that any of the symptoms, such as the tumor, metastasis thereof, the vascularization of the

tumors or other parameters by which the disease is characterized are reduced, ameliorated, prevented, placed in a state of remission, or maintained in a state of remission. It also means that the hallmarks of neoplastic disease and metastasis may be eliminated, reduced or prevented by the treatment. Non-limiting examples of the hallmarks include uncontrolled degradation of the basement membrane and proximal extracellular matrix, migration, division, and organization of the endothelial cells into new functioning capillaries, and the persistence of such functioning capillaries.

[0158] As used herein, operatively linked or operationally associated refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, e.g., Kozak *J. Biol. Chem.* 266:19867-19870 (1991)) can be inserted immediately 5' of the start codon and may enhance expression. The desirability of (or need for) such modification may be empirically determined.

[0159] As used herein, pharmaceutically acceptable salts, esters or other derivatives of the conjugates include any salts, esters or derivatives that may be readily prepared by those of skill in this art using known methods for such derivatization and that produce compounds that may be administered to animals or humans without substantial toxic effects and that either are pharmaceutically active or are prodrugs.

[0160] As used herein, a prodrug is a compound that, upon in vivo administration, is metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the compound. To produce a prodrug, the pharmaceutically active compound is modified such that the active compound will be regenerated by metabolic processes. The prodrug may be designed to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism in vivo, those of skill in this art, once a pharmaceutically active compound is known, can design prodrugs of the compound (see, e.g., Nogrady (1985) *Medicinal Chemistry A Biochemical Approach*, Oxford University Press, New York, pages 388-392).

[0161] As used herein, a drug identified by the screening methods provided herein refers to any compound that is a candidate for use as a therapeutic or as lead compound for designed a therapeutic. Such compounds can be small molecules, including small organic molecules, peptides, peptide mimetics, antisense molecules, antibodies, fragments of antibodies, recombinant antibodies and other such compound which can serve as drug candidate or lead compound.

[0162] As used herein, production by recombinant means by using recombinant DNA methods means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA.

[0163] As used herein, a promoter region or promoter element refers to a segment of DNA or RNA that controls transcription of the DNA or RNA to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to as the promoter. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences may be cis acting or may be responsive to trans acting factors. Promoters, depending upon the nature of the regulation, may be constitutive or regulated. Exemplary promoters contemplated for use in prokaryotes include the bacteriophage T7 and T3 promoters.

[0164] As used herein, a receptor refers to a molecule that has an affinity for a given ligand. Receptors may be naturally-occurring or synthetic molecules. Receptors may also be referred to in the art as anti-ligands. As used herein, the receptor and anti-ligand are interchangeable. Receptors can be used in their unaltered state or as aggregates with other species. Receptors may be attached, covalently or noncovalently, or in physical contact with, to a binding member, either directly or indirectly via a specific binding substance or linker. Examples of receptors, include, but are not limited to: antibodies, cell membrane receptors surface receptors and internalizing receptors, monoclonal antibodies and antisera reactive with specific antigenic determinants [such as on viruses, cells, or other materials], drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes, and organelles.

[0165] Examples of receptors and applications using such receptors, include but are not restricted to:

[0166] a) enzymes: specific transport proteins or enzymes essential to survival of microorganisms, which could serve as targets for antibiotic [ligand] selection;

[0167] b) antibodies: identification of a ligand-binding site on the antibody molecule that combines with the epitope of an antigen of interest may be investigated; determination of a sequence that mimics an antigenic epitope may lead to the development of vaccines of which the immunogen is based on one or more of such sequences or lead to the development of related diagnostic agents or compounds useful in therapeutic treatments such as for auto-immune diseases

[0168] c) nucleic acids: identification of ligand, such as protein or RNA, binding sites;

[0169] d) catalytic polypeptides: polymers, preferably polypeptides, that are capable of promoting a chemical reaction involving the conversion of one or more reactants to one or more products; such polypeptides generally include a binding site specific for at least one reactant or reaction intermediate and an active functionality proximate to the binding site, in which the functionality is capable of chemically modifying the bound reactant [see, e.g., U.S. Pat. No. 5,215,899];

[0170] e) hormone receptors: determination of the ligands that bind with high affinity to a receptor is useful in the

development of hormone replacement therapies; for example, identification of ligands that bind to such receptors may lead to the development of drugs to control blood pressure; and

[0171] f) opiate receptors: determination of ligands that bind to the opiate receptors in the brain is useful in the development of less-addictive replacements for morphine and related drugs.

[0172] As used herein, sample refers to anything which may contain an analyte for which an analyte assay is desired. The sample may be a biological sample, such as a biological fluid or a biological tissue. Examples of biological fluids include urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus, amniotic fluid or the like. Biological tissues are aggregate of cells, usually of a particular kind together with their intercellular substance that form one of the structural materials of a human, animal, plant, bacterial, fungal or viral structure, including connective, epithelium, muscle and nerve tissues. Examples of biological tissues also include organs, tumors, lymph nodes, arteries and individual cell(s).

[0173] As used herein: stringency of hybridization in determining percentage mismatch is as follows:

[0174] 1) high stringency: 0.1×SSPE, 0.1% SDS, 65° C.

[0175] 2) medium stringency: 0.2×SSPE, 0.1% SDS, 50° C.

[0176] 3) low stringency: 1.0×SSPE, 0.1% SDS, 50° C.

[0177] Those of skill in this art know that the washing step selects for stable hybrids and also know the ingredients of SSPE (see, e.g., Sambrook, E. F. Fritsch, T. Maniatis, in: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989), vol. 3, p. B.13, see, also, numerous catalogs that describe commonly used laboratory solutions). SSPE is pH 7.4 phosphate-buffered 0.18 NaCl. Further, those of skill in the art recognize that the stability of hybrids is determined by  $T_m$ , which is a function of the sodium ion concentration and temperature ( $T_m=81.5^\circ\text{C}.-16.6(\log_{10}[\text{Na}^+])+0.41(\%G+C)-600/1$ ), so that the only parameters in the wash conditions critical to hybrid stability are sodium ion concentration in the SSPE (or SSC) and temperature.

[0178] It is understood that equivalent stringencies may be achieved using alternative buffers, salts and temperatures. By way of example and not limitation, procedures using conditions of low stringency are as follows (see also Shilo and Weinberg, *Proc. Natl. Acad. Sci. USA*, 78:6789-6792 (1981)): Filters containing DNA are pretreated for 6 hours at 40° C. in a solution containing 35% formamide, 5×SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA (10×SSC is 1.5 M sodium chloride, and 0.15 M sodium citrate, adjusted to a pH of 7).

[0179] Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20×10<sup>6</sup> cpm <sup>32</sup>P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 40° C., and then washed for 1.5 hours at 55° C. in a solution containing 2×SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced

with fresh solution and incubated an additional 1.5 hours at 60° C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68° C. and reexposed to film. Other conditions of low stringency which may be used are well known in the art (e.g., as employed for cross-species hybridizations).

[0180] By way of example and not way of limitation, procedures using conditions of moderate stringency is provided. For example, but not limited to, procedures using such conditions of moderate stringency are as follows: Filters containing DNA are pretreated for 6 hours at 55° C. in a solution containing 6×SSC, 5×Denhart's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution and 5-20×10<sup>6</sup> cpm <sup>32</sup>P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 55° C., and then washed twice for 30 minutes at 60° C. in a solution containing 1×SSC and 0.1% SDS. Filters are blotted dry and exposed for autoradiography. Other conditions of moderate stringency which may be used are well-known in the art. Washing of filters is done at 37° C. for 1 hour in a solution containing 2×SSC, 0.1% SDS.

[0181] By way of example and not way of limitation, procedures using conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65° C. in buffer composed of 6×SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 hours at 65° C. in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20×10<sup>6</sup> cpm of <sup>32</sup>P-labeled probe. Washing of filters is done at 37° C. for 1 hour in a solution containing 2×SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1×SSC at 50° C. for 45 minutes before autoradiography. Other conditions of high stringency which may be used are well known in the art.

[0182] The term substantially identical or homologous or similar varies with the context as understood by those skilled in the relevant art and generally means at least 70%, preferably means at least 80%, more preferably at least 90%, and most preferably at least 95% identity.

[0183] As used herein, substantially identical to a product means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place of the product.

[0184] As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound may, however, be a mixture of stereoisomers or isomers. In such instances, further purification might increase the specific activity of the compound.

[0185] As used herein, target cell refers to a cell that expresses an MTSP in vivo.

**[0186]** As used herein, test substance refers to a chemically defined compound (e.g., organic molecules, inorganic molecules, organic/inorganic molecules, proteins, peptides, nucleic acids, oligonucleotides, lipids, polysaccharides, saccharides, or hybrids among these molecules such as glycoproteins, etc.) or mixtures of compounds (e.g., a library of test compounds, natural extracts or culture supernatants, etc.) whose effect on an MTSP, particularly a single chain form that includes the protease domain or a sufficient portion thereof for activity, as determined by in vitro method, such as the assays provided herein.

**[0187]** As used herein, the terms a therapeutic agent, therapeutic regimen, radioprotectant, chemotherapeutic mean conventional drugs and drug therapies, including vaccines, which are known to those skilled in the art. Radiotherapeutic agents are well known in the art.

**[0188]** As used herein, treatment means any manner in which the symptoms of a conditions, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein.

**[0189]** As used herein, vector (or plasmid) refers to discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof. The vectors typically remain episomal, but may be designed to effect integration of a gene or portion thereof into a chromosome of the genome. Also contemplated are vectors that are artificial chromosomes, such as yeast artificial chromosomes and mammalian artificial chromosomes. Selection and use of such vehicles are well known to those of skill in the art. An expression vector includes vectors capable of expressing DNA that is operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

**[0190]** As used herein, protein binding sequence refers to a protein or peptide sequence that is capable of specific binding to other protein or peptide sequences generally, to a set of protein or peptide sequences or to a particular protein or peptide sequence.

**[0191]** As used herein, epitope tag refers to a short stretch of amino acid residues corresponding to an epitope to facilitate subsequent biochemical and immunological analysis of the epitope tagged protein or peptide. Epitope tagging is achieved by appending the sequence of the epitope tag to the protein-encoding sequence in an appropriate expression vector. Epitope tagged proteins can be affinity purified using highly specific antibodies raised against the tags.

**[0192]** As used herein, metal binding sequence refers to a protein or peptide sequence that is capable of specific binding to metal ions generally, to a set of metal ions or to a particular metal ion.

**[0193]** As used herein, a composition refers to a any mixture. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

**[0194]** As used herein, a combination refers to any association between two or among more items.

**[0195]** As used herein, fluid refers to any composition that can flow. Fluids thus encompass compositions that are in the form of semi-solids, pastes, solutions, aqueous mixtures, gels, lotions, creams and other such compositions.

**[0196]** As used herein, a cellular extract refers to a preparation or fraction which is made from a lysed or disrupted cell.

**[0197]** As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of a protein alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism.

**[0198]** As used herein, an agent is the to be rationally selected or designed when the agent is chosen on a non-random basis which takes into account the sequence of the target site and/or its conformation in connection with the agent's action. As described in the Examples, there are proposed binding sites for serine protease and (catalytic) sites in the protein having SEQ ID NO:3 or SEQ ID NO:4. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up these sites. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to the ATP or calmodulin binding sites or domains.

**[0199]** For clarity of disclosure, and not by way of limitation, the detailed description is divided into the subsections that follow.

**[0200]** B. MTSP Proteins, Muteins, Derivatives and Analogs Thereof

**[0201]** MTSPs

**[0202]** The MTSPs are a family of transmembrane serine proteases that are found in mammals and also other species that share a number of common structural features including: a proteolytic extracellular C-terminal domain; a transmembrane domain, with a hydrophobic domain near the N-terminus; a short cytoplasmic domain; and a variable length stem region containing modular domains. The proteolytic domains share sequence homology including conserved his, asp, and ser residues necessary for catalytic activity that are present in conserved motifs. The MTSPs are synthesized as zymogens, and activated to double chain forms by cleavage. It is shown herein that the single chain proteolytic domain can function in vitro and, hence is useful in in vitro assays for identifying agents that modulate the activity of members of this family. Also provided are family members designated MTSP3, MTSP4 and an MTSP6 variant.

**[0203]** The MTSP family is a target for therapeutic intervention and also some, may serve as diagnostic markers for tumor development, growth and/or progression. As discussed, the members of this family are involved in proteolytic processes that are implicated in tumor development, growth and/or progression. This implication is based upon their functions as proteolytic enzymes in processes related to ECM degradative pathways. In addition, their levels of expression or level of activation or their apparent activity

resulting from substrate levels or alterations in substrates and levels thereof differs in tumor cells and non-tumor cells in the same tissue. Hence, protocols and treatments that alter their activity, such as their proteolytic activities and roles in signal transduction, and/or their expression, such as by contacting them with a compound that modulates their activity and/or expression, could impact tumor development, growth and/or progression. Also, in some instances, the level of activation and/or expression may be altered in tumors, such as lung carcinoma, colon adenocarcinoma and ovarian carcinoma.

**[0204]** The MTSP may serve as a diagnostic marker for tumors. It is shown herein, that MTSP3 and MTSP4 and the MTSP6 variant provided herein are expressed and/or activated in certain tumors; hence their activation or expression may serve as a diagnostic marker for tumor development, growth and/or progression. In other instances the MTSP protein can exhibit altered activity by virtue of a change in activity or expression of a co-factor therefor or a substrate therefor. In addition, in some instances, these MTSPs and/or variants thereof may be shed from cell surfaces. Detection of the shed MTSPs, particularly the extracellular domains, in body fluids, such as serum, blood, saliva, cerebral spinal fluid, synovial fluid and interstitial fluids, urine, sweat and other such fluids and secretions, may serve as a diagnostic tumor marker. In particular, detection of higher levels of such shed polypeptides in a subject compared to a subject known not to have any neoplastic disease or compared to earlier samples from the same subject, can be indicative of neoplastic disease in the subject.

**[0205]** Provided herein are isolated substantially pure single polypeptides that contain the protease domain of an MTSP as a single chain. The MTSPs contemplated herein are not expressed on endothelial cells, and, preferably, are expressed on tumor cells, typically at a level that differs from the level in which they are expressed in the non-tumor cell of the same type. Hence, for example, if the MTSP is expressed in an ovarian tumor cell, to be of interest herein with respect to ovarian cancer, it is expressed at the same level in non-tumor ovarian cells. MTSP protease domains include the single chain protease domains of MTSP1, MTSP3, MTSP4, MTSP6 and other such proteases, including, but are not limited to, corin, enterpeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRS2, and TMPRSS4.

**[0206]** Provided are the protease domains or proteins that include a portion of an MTSP that is the protease domain of any MTSP, particularly an MTSP1, MTSP3, MTSP4 and MTSP6. The protein can also include other non-MTSP sequences of amino acids, but will include the protease domain or a sufficient portion thereof to exhibit catalytic activity in any in vitro assay that assess such protease activity, such as any provided herein.

**[0207]** Also provided herein are nucleic acid molecules that encode MTSP proteins and the encoded proteins. In particular, nucleic acid molecules encoding MTSP-3 and MTSP-4 from animals, including splice variants thereof are provided. The encoded proteins are also provided. Also provided are functional domains thereof.

**[0208]** In specific aspects, the MTSP protease domains, portions thereof, and muteins thereof are from or based on animal MTSPs, including, but are not limited to, rodent,

such as mouse and rat; fowl, such as chicken; ruminants, such as goats, cows, deer, sheep; ovine, such as pigs; and humans.

**[0209]** In particular, MTSP derivatives can be made by altering their sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other nucleic sequences which encode substantially the same amino acid sequence as a MTSP gene can be used. These include but are not limited to nucleotide sequences comprising all or portions of MTSP genes that are altered by the substitution of different codons that encode the amino acid residue within the sequence, thus producing a silent change. Likewise, the MTSP derivatives include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of MTSP, including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid (see, e.g., Table 1).

**[0210]** In a preferred embodiment, the substantially purified MTSP protease is encoded by a nucleic acid that hybridizes to the a nucleic acid molecule containing the protease domain encoded by the nucleotide sequence set forth in any of SEQ. ID Nos. 1, 3, 5, 7, 9 or 11 under at least moderate, generally high, stringency conditions, such that the protease domain encoding nucleic acid thereof hybridizes along its full length. In preferred embodiments the substantially purified MTSP protease is a single chain polypeptide that includes substantially the sequence of amino acids set forth in any SEQ ID Nos. 2, 4, 6, 8, 10 and 12 that encodes the protease domain. Specific sequences for the following human MTSPs and domains thereof are provided as follows: SEQ ID No. 3 MTSP3 nucleic acid sequence; SEQ ID No. 4 MTSP3 amino acid sequence; SEQ ID No. 5 MTSP4 nucleic acid encoding the protease domain; SEQ ID No. 6 MTSP4 amino acid sequence of the protease domain; SEQ ID No. 7 MTSP4-L nucleic acid sequence; SEQ ID No.8 MTSP4-L amino acid sequence; SEQ ID No. 9 MTSP4-S nucleic acid sequence; SEQ ID No. 10 MTSP4-S amino acid sequence; SEQ ID No. 11 MTSP6 nucleic acid sequence; SEQ ID No. 12 MTSP6 amino acid sequence. SEQ ID No. 1 sets forth the nucleic acid sequence of the long form of MTSP1; SEQ ID No. 2 the encoded amino acid sequence; SEQ ID No. 49 sets forth the sequence of a protease domain of an MTSP1, and SEQ ID No. 50 the sequence of the encoded single chain protease domain thereof. FIGS. 1-3 depict the structural organization of the MTSP3, MTSP4 and MTSP6, respectively.

**[0211]** In particular, exemplary protease domains include at least a sufficient portion of sequences of amino acids set

forth as amino acids 615-855 in SEQ ID No. 2 (encoded by nucleotides 1865-2587 in SEQ ID No. 1; see also SEQ ID Nos. 49 and 50) from MTSP1 (matriptase), amino acids 205-437 of SEQ ID NO. 4 from MTSP3, SEQ ID No. 6, which sets forth the protease domain of MTSP4, and amino acids 217-443 of SEQ ID No. 11 from MTSP6.

[0212] Also contemplated are nucleic acid molecules that encode a single chain MTSP protease that have proteolytic activity in an in vitro proteolysis assay and that have at least 60%, 70%, 80%, 85%, 90% or 95% sequence identity with the full length of a protease domain of an MTSP protein, or that hybridize along their full length to a nucleic acids that encode a protease domain, particularly under conditions of moderate, generally high, stringency. As above, the encoded polypeptides contain the protease as a single chain.

[0213] The isolated nucleic acids may include of at least 8 nucleotides of an MTSP sequence. In other embodiments, the nucleic acids may contain least 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of a MTSP sequence, or a full-length MTSP coding sequence. In another embodiment, the nucleic acids are smaller than 35, 200 or 500 nucleotides in length. Nucleic acids can be single or double stranded. Nucleic acids that hybridizes to or complementary to the foregoing sequences, in particular the inverse complement to nucleic acids that hybridizes to the foregoing sequences (i.e., the inverse complement of a nucleic acid strand has the complementary sequence running in reverse orientation to the strand so that the inverse complement would hybridize without mismatches to the nucleic acid strand; thus, for example, where the coding strand is that hybridizes to a nucleic acid with no mismatches between the coding strand and the that hybridizes strand, then the inverse complement of the that hybridizes strand is identical to the coding strand) are also provided. In specific aspects, nucleic acids are provided that include a sequence complementary to (specifically are the inverse complement of) at least 10, 25, 50, 100, or 200 nucleotides or the entire coding region of an MTSP encoding nucleic acid, particularly the protease domain thereof. For MTSP3 and MTSP4 the full-length protein or domain or active fragment thereof.

[0214] For each of the nucleic acid molecules, the nucleic acid can be DNA or RNA or PNA or other nucleic acid analogs or may include non-natural nucleotide bases.

[0215] Also provided are isolated nucleic acid molecules that include a sequence of nucleotides complementary to the nucleotide sequence encoding an MTSP.

[0216] Probes and primers derived from the nucleic acid molecules are provided, Such probes and primers contain at least 8, 14, 16, 30, 100 or more contiguous nucleotides with identity to contiguous nucleotides of an MTSP, including, but are not limited to, MTSP1, MTSP3, MTSP4 and MTSP6. The probes and primers are optionally labelled with a detectable label, such as a radiolabel or a fluorescent tag, or can be mass differentiated for detection by mass spectrometry or other means.

[0217] Plasmids and vectors containing the nucleic acid molecules are also provided. Cells containing the vectors, including cells that express the encoded proteins are provided. The cell can be a bacterial cell, a yeast cell, a fungal cell, a plant cell, an insect cell or an animal cell. Methods for

producing an MTSP or single chain form of the protease domain thereof by, for example, growing the cell under conditions whereby the encoded MTSP is expressed by the cell, and recovering the expressed protein, are provided herein. As noted, for MTSP3 and MTSP4, the full-length zymogens and activated proteins and activated (two strand) protease and single chain protease domains are provided.

[0218] Except for the MTSP proteins (MTSP3 and MTSP4) heretofore unidentified and provided herein, the isolated polypeptides contain the MTSP protease domain or a catalytically active portion thereof and, generally, do not contain additional MTSP. Hence isolated, substantially pure proteases that e the protease domains or catalytically active portion thereof in single chain form of MTSPs are provided. The protease domains may be included in a longer protein, but such longer protein is not the MTSP zymogen.

[0219] Thus, MTSP3 and MTSP4 proteins are provided. For these proteins, the domains, fragments, derivatives or analogs that are functionally active, i.e., capable of exhibiting one or more functional activities associated with the MTSP protein, e.g., serine protease activity, immunogenicity and antigenicity, are provided. As discussed above, the protease domains thereof are also provided. For MTSP3 and MTSP4, the zymogens and activated forms, and also, the single chain and double chain, activated protease domains are provided.

[0220] Also provided are nucleic acid molecules that hybridize to the above-noted sequences of nucleotides encoding MTSP3 and MTSP4 (SEQ ID Nos. 3, 5, 7 and 9) at least at low stringency, more preferably at moderate stringency, and most preferably at high stringency, and that encode the protease domain and/or the full length protein or other domains of an MTSP family member, such as MTSP3, MTSP4, MTSP6 or a splice variant or allelic variant thereof, or MTSP6 or a splice variant or allelic variant thereof. Preferably the molecules hybridize under such conditions along their full length for at least one domain and encode at least one domain, such as the protease or extracellular domain, of the polypeptide. In particular, such nucleic acid molecules include any isolated nucleic fragment that encodes at least one domain of a membrane serine protease, that (1) contains a sequence of nucleotides that encodes the protease or a domain thereof, and (2) is selected from among:

[0221] (a) a sequence of nucleotides that encodes the protease or a domain thereof includes a sequence of nucleotides set forth above;

[0222] (b) a sequence of nucleotides that encodes such portion or the full length protease and hybridizes under conditions of high stringency, preferably to nucleic acid that is complementary to a mRNA transcript present in a mammalian cell that encodes such protein or fragment thereof;

[0223] (c) a sequence of nucleotides that encodes a transmembrane protease or domain thereof that includes a sequence of amino acids encoded by such portion or the full length open reading frame; and

[0224] (d) a sequence of nucleotides that encodes the transmembrane protease that includes a sequence of amino acids encoded by a sequence of nucleotides that encodes such subunit and hybridizes under con-

ditions of high stringency to DNA that is complementary to the mRNA transcript.

**[0225]** Exemplary MTSPs

**[0226]** The above discussion provides an overview and some details of the exemplified MTSPs. The following discussion provides additional details (see, also, EXAMPLES).

**[0227]** MTSP1 (matriptase)

**[0228]** Matriptase is a trypsin-like serine protease with broad spectrum cleavage activity and two potential regulatory modules. It was named "matriptase" because its ability to degrade the extra-cellular matrix and its trypsin-like activity. When isolated from breast cancer cells (or T-47D cell conditioned medium), matriptase has been reported to be primarily in an uncomplexed form. Matriptase has been isolated from human milk; when isolated from human milk, matriptase was reported to be in one of two complexed forms, 95 kDa (the predominant form) and 110 kDa; uncomplexed matriptase was not detected. (Liu, et al., *J. Biol. Chem.* 274(26):18237-18242 (1999).) It has been proposed that matriptase exists as an uncomplexed protease when in its active state. In breast milk, matriptase has been reported to exist in complex with a fragment of hepatocyte growth factor inhibitor-1 (HAI-1), a Kuntz-type serine protease inhibitor having activity against trypsin-like serine proteases.

**[0229]** Ecotin and Ecotin M84R/M85R are macromolecular inhibitors of serine proteases of the chymotrypsin fold and inhibit ductal branching, morphogenesis and differentiation of the explanted ductal prostate. PC-3 is a cell line derived from prostate cancer epithelial cells. Ecotin and M84R/M85R ecotin were found to decrease tumor size and metastasis in PC-3 implanted nude mice.

**[0230]** Matriptase has been isolated and its encoding nucleic acids cloned from T-47D human breast cancer cell-conditioned medium (Lin et al. (1999) *J. Biol. Chem.* 274:18231-18236). Upon analysis of the cDNA, it was determined that the full length protease has 683 amino acids and contains three main structural regions: a serine protease domain near the carboxyl-terminal region, four tandem low-density lipoprotein receptor domains, and two tandem complement subcomponents C1r and C1s.

**[0231]** Studies to identify additional serine proteases made by cancer cells were done using PC-3 cells. A serine protease termed "MT-SP1", reported to be a transmembrane protease was cloned (Takeuchi et al. (1999) *Proc. Natl. Acad. Sci. U.S.A.* 96:11054-11061). It was subsequently found the originally identified matriptase sequence is included in the translated sequence of the cDNA that encodes MT-SP1. The matriptase cDNA was reported to be a partial MT-SP1 cDNA and to lack 516 of the coding nucleotides (Takeuchi, et al., *J. Biol. Chem.* 275:26333-26342 (2000).) Since the reported matriptase encoding cDNA sequence encoded a possible initiating methionine, it was proposed that alternative splicing could yield a protein lacking the N-terminal region of MTSP1.

**[0232]** Matriptase and MT-SP1 demonstrate trypsin-like protease activity and are Type II transmembrane proteins with a common extracellular protease domain. Studies of substrate specificity of MT-SP1 reveal that protease-acti-

vated receptor 2 (PAR2) and single-chain urokinase-type plasminogen activator (sc-uPA) are macromolecular substrates of MT-SP1. PAR2 is functions in inflammation, cytoprotection and/or cell adhesion, while sc-uPa is functions in tumor cell invasion and metastasis.

**[0233]** An exemplary nucleotide sequences encoding a human MTSP1 is set forth in SEQ ID Nos 1 and 2 (see also SEQ ID Nos. 49 and 50 for the protease domain thereof). As previously noted SEQ ID No. 1 sets for an MTSP1-encoding nucleic acid sequence. This sequence is the longer version and includes the protease domain, which is common to both variants Nucleic acids encoding the MTSP that hybridizes to the nucleotide sequence set forth in SEQ ID No. 1 can be obtained by any method known in the art, e.g., by PCR amplification using synthetic primers that hybridizes to the 3' and 5' ends of the sequence and/or by cloning from a cDNA or genomic library using a PCR amplification product or an oligonucleotide specific for the gene sequence (e.g., as described in Section C herein). Homologs (e.g., nucleic acids of the above-listed genes of species other than human) or other related sequences (e.g., paralogs) and muteins can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular sequence provided as a probe using methods well known in the art for nucleic acid hybridization and cloning.

**[0234]** Isolated single chain protease domains of MTSP1 proteins from animals are provided herein. As shown herein, the single chain protease domain is catalytically active and can be used in a variety of drug screening assays, particularly in vitro proteolytic assays. Exemplary MTSP protease domains are set forth as the amino acids (615-855 of SEQ ID No. 2) encoded by nucleotides 1865-2587 of SEQ ID No. 1 (see, also, SEQ ID Nos. 49 and 50). The MTSP1 single chain protease domain is catalytically active

**[0235]** Muteins of the MTSP1 proteins are provided. In the activated double chain molecule, residue 731 forms a disulfide bond with the Cys at residue 604. In the single chain form, the residue at 731 in the protease domain is free. Muteins in which Cys residues, particularly the free Cys residue (amino acid 731 in SEQ ID No. 2) in the single chain protease domain are provided. Other muteins in which conservative amino acids replacements are effected and that retain proteolytic activity as a single chain are also provided. Such changes may be systematically introduced and tested for activity in in vitro assays, such as those provided herein.

**[0236]** MTSP3

**[0237]** In a specific embodiment, a nucleic acid that encodes a MTSP, designated MTSP3 is provided. In particular, the nucleic acid includes an open reading frame within the following sequence of nucleotides set forth in SEQ ID No. 3. In particular the protein is encoded by the open reading frame that begins at nucleotide 261 and ends at 1574.

**[0238]** Also provided are nucleic acid molecules that hybridize under conditions of at least low stringency, preferably moderate stringency, more preferably high stringency to the following sequence of nucleic acids (SEQ ID No. 3), particularly to the open reading frame encompassed by nucleotides that encode a single protease domain thereof, or any domain of MTSP3

**[0239]** Also included are substantially purified MTSP3 zymogen, activated double chains, single chain protease

domains and double chain protease domains. These are encoded by a nucleic acid that includes sequence encoding a protease domain that exhibits proteolytic activity and that hybridizes to a nucleic acid molecule having a nucleotide sequence set forth in SEQ ID No. 3, typically under moderate, generally under high stringency, conditions and most preferably along the full length of the protease domain. Splice variants are also contemplated herein.

[0240] In a preferred embodiment, the isolated nucleic acid fragment hybridizes to the nucleic acid having the nucleotide sequence set forth in SEQ ID No: 3 under high stringency conditions, and preferably comprises the sequence of nucleotides set forth in any of SEQ ID Nos. 3 or comprises a portion thereof that encodes a transmembrane domain and may additionally include a LDLR domain, a scavenger-receptor cysteine rich (SRCR) domain and a serine protease catalytic domain or any other identified domain (see FIGURES) or comprises nucleic acid molecule that encodes the protein encoded by SEQ ID NO. 4.

[0241] The isolated nucleic acid fragment is DNA, including genomic or cDNA, or is RNA, or can include other components, such as protein nucleic acid. The isolated nucleic acid may include additional components, such as heterologous or native promoters, and other transcriptional and translational regulatory sequences, these genes may be linked to other genes, such as reporter genes or other indicator genes or genes that encode indicators.

[0242] Also provided is an isolated nucleic acid molecule that includes the sequence of molecules that is complementary to the nucleotide sequence encoding the MTSP or the portion thereof.

[0243] Also provided are fragments thereof that can be used as probes or primers and that contain at least about 10 nucleotides, more preferably 14 nucleotides, more preferably at least about 16 nucleotides, most preferably at least about 30 nucleotides.

[0244] Hence provided herein are polypeptides that are encoded by such nucleic acid molecules. Included among those polypeptides are the MTSP3 protease domain or a polypeptide with conservative amino acid changes such that the specificity and protease activity remains substantially unchanged. In particular, a substantially purified mammalian MTSP protein is provided that has a transmembrane domain and may additionally include a CUB domain, one or more of an LDLR domain(s), a scavenger-receptor cysteine rich (SRCR) domain and a serine protease catalytic domain is provided.

[0245] Also provided is a substantially purified protein comprising a sequence of amino acids that has at least 60%, more preferably at least about 90%, most preferably at least about 95%, identity to the MTSP3, wherein the percentage identity is determined using standard algorithms and gap penalties that maximize the percentage identity. The human MTSP3 protein is most preferred, although other mammalian MTSP3 proteins are contemplated.

[0246] Muteins of MTSP3, particularly those in which Cys residues, such as the Cys310 in the single chain protease domain, is replaced with another amino acid that does not eliminate the activity, are provided.

[0247] MTSP4

[0248] Among the proteins provided herein is MTSP4. MTSP4 is highly expressed in the liver, and is expressed in

substantially lower levels in other tissues (see, EXAMPLES). It is also expressed in non-liver-derived tumors (see EXAMPLES), including Burkitt's lymphoma, colorectal adenocarcinoma (SW480), lung carcinoma (A549), and in leukemic cells, indicating a role in one or more of tumor progression, tumor invasion, tumor growth and tumor metastases.

[0249] Also provided are nucleic acid molecules that hybridize under conditions of at least low stringency, preferably moderate stringency, more preferably high stringency to the sequence of nucleic acids set forth in SEQ ID Nos. 5, 7 or 9), particularly to the open reading frame encompassed by nucleotides that encode a single protease domain thereof, or any domain of an MTSP4.

[0250] Also included are substantially purified MTSP4 zymogens, activated double chains, single chain protease domains and double chain protease domains. These are encoded by a nucleic acid that includes sequence encoding a protease domain that exhibits proteolytic activity and that hybridizes to a nucleic acid molecule having a nucleotide sequence set forth in SEQ ID Nos. 5, 7 and 9, typically under moderate, generally under high stringency, conditions and most preferably along the full length of the protease domain.

[0251] In a preferred embodiment, the isolated nucleic acid fragment hybridizes to the nucleic acid having the nucleotide sequence set forth in SEQ ID No: 5, 7 or 9 under high stringency conditions, and preferably comprises the sequence of nucleotides set forth in any of SEQ ID Nos. 5, 7 or 9 comprises a portion thereof that encodes a transmembrane domain and may additionally include a LDLR domain, a scavenger-receptor cysteine rich (SRCR) domain and a serine protease catalytic domain or any other identified domain (see FIGURES) or comprises nucleic acid molecule that encodes the protein encoded by SEQ ID NO. 6, 9 or 10.

[0252] The isolated nucleic acid fragment is DNA, including genomic or cDNA, or is RNA, or can include other components, such as protein nucleic acid. The isolated nucleic acid may include additional components, such as heterologous or native promoters, and other transcriptional and translational regulatory sequences, these genes may be linked to other genes, such as reporter genes or other indicator genes or genes that encode indicators.

[0253] Also provided is an isolated nucleic acid molecule that includes the sequence of molecules that is complementary to the nucleotide sequence encoding and MTSP4 or the portion thereof.

[0254] Also provided are fragments thereof that can be used as probes or primers and that contain at least about 10 nucleotides, more preferably 14 nucleotides, more preferably at least about 16 nucleotides, most preferably at least about 30 nucleotides.

[0255] In particular nucleic acid molecules encoding two forms of MTSP4 are provide. The encoded proteins are multi-domain, type II membrane-type serine proteases and include a transmembrane domain at the N terminus followed by a CUB domain, 3 LDLR domains and a trypsin-like serine protease domain at the C terminus. The difference between the two forms, which are splice variants, is the absence in MTSP4-S of a 432-bp nucleotide sequence

between the transmembrane and the CUB domains (see FIG. 2; see, also SEQ ID Nos. 5-10).

[0256] Also provided is a nucleic acid that encodes the extracellular protease domain of an MTSP4 is provided. Both forms of MTSP4 exemplified herein include a protease domain in common (see SEQ ID Nos. 5 and 6).

[0257] In particular, the extracellular protease domain of the MTSP4 proteins is encoded by the open reading frame that begins at nucleotide 1 and ends at 708 (TGA) (SEQ ID No. 5). This open reading frame encodes a portion of the MSTP4 protein and includes the protease domain. Full length MSTP4 proteins (SEQ ID Nos. 7 and 9) include the above domain. The extracellular protease domain, as a single chain, and also an activated double chain, exhibit protease activity. The disulfide bonds that form that two chain form of MTSP forms are likely between Cys415 and Cys535 for MTSP4-S, and between Cys559 and Cys679 for MTSP4-L.

[0258] For use of the single chain protease domain thereof, it is of interest to replace the free Cys (i.e. Cys535 (Cys679)) in the protease domain with another amino acid, such as any amino acid that does not alter the function (such change is likely to be any amino acid). Thus, muteins of MTSP4, particularly those in which Cys residues, such as the Cys535 and Cys679 in the single chain protease domains of MTSP4-S and MTSP4-L, respectively, are provided.

#### [0259] MTSP6

[0260] Nucleic acid and the encoded MTSP6 protein of an exemplary MTSP6 are also provided. The respective sequences are set forth in SEQ ID Nos. 11 and 12. The MTSP6 DNA and protein sequences were analyzed using DNA Strider (version 1.2). The ORF encoding the MTSP6 variant provided herein is composed of 1,362 bp, which translate into a 453-amino acid protein. MTSP6 is a multi-domain, type-II membrane-type serine protease containing a transmembrane domain (amino acids 48-68) at the N-terminus followed by a LDLRa domain (LDL receptor domain class a) (amino acids 72-108), a SR domain (Scavenger receptor Cys-rich domain)(amino acids 109-205), and a trypsin-like serine protease domain (amino acids 216-443) (see FIG. 3). Muteins of MTSP6, particularly those in which Cys residues, such as the Cys324 in the single chain protease domain of MTSP6 are provided.

[0261] International PCT application No. WO 00/52044 describes MTSPs that resemble the MTSP6 provided herein. The polypeptide provided therein differs at single amino acid positions, such as 90 in SEQ ID No. 12 (Ala is replaced with a Thr), and significantly from the instant MTSP6 in that ten amino acids (amino acid nos. 46-55 in SEQ ID No. 12) are replaced with the eleven amino acids: phe glu val phe ser gin ser ser ser leu gly (SEQ ID No. 59) resulting in a protein that is one 454 amino acids long.

[0262] There are a few other amino acid sequence differences and a number of nucleic acid sequence differences. Significantly, there are substantial differences in the protease domain at amino acids 368-394 (368 ICNHRDVYGGI-ISPMLCAGYLTGGVD----394; SEQ ID No. 12) are replaced at position 369-396 with amino acids:

[0263] 369 DLQPQ----GRVRWHHLPLHALRGLPDG-WRWN 396, where the differences from 368-394 (Seq ID No. 12) are indicated.

[0264] In addition, a second C-terminus truncated variant with an altered protease domain is identified in the PCT application. The variant is the same as the 454 variant through amino acid 261 thereof (corresponding to 160 of SEQ ID No. 12 herein), followed by 33 amino acids (see SEQ ID No. 60 herein) that differ by virtue of a frame shift.

#### [0265] C. Tumor Specificity and Tissue Expression Profiles

[0266] Each MTSP has a characteristic tissue expression profile; the MTSPs in particular, although not exclusively expressed or activated in tumors, exhibit characteristic tumor tissue expression or activation profiles. In some instances, MTSPs may have different activity in a tumor cell from a non-tumor cell by virtue of a change in a substrate or cofactor therefor or other factor that would alter the apparent of functional activity of the MTSP. Hence each can serve as a diagnostic marker for particular tumors, by virtue of a level of activity and/or expression or function in a subject (i.e. a mammal, particularly a human) with neoplastic disease, compared to a subject or subjects that do not have the neoplastic disease. In addition, detection of activity (and/or expression) in a particular tissue can be indicative of neoplastic disease. Shed MTSPs in body fluids can be indicative of neoplastic disease. Also, by virtue of the activity and/or expression profiles of each, they can serve as therapeutic targets, such as by administration of modulators of the activity thereof, or, as by administration of a prodrug specifically activated by one of the MTSPs.

#### [0267] Tissue Expression Profiles

#### [0268] MTSP3

[0269] The MTSP3 transcript was detected in lung carcinoma (LX-1), colon adenocarcinoma (CX-1), colon adenocarcinoma (GI-112) and ovarian carcinoma (GI-102). No apparent signal was detected in another form of lung carcinoma (GI-117), breast carcinoma (GI-101), pancreatic adenocarcinoma (GI-103) and prostatic adenocarcinoma (PC3). MTSP1 is expressed in breast cancers.

#### [0270] MTSP4

[0271] The MTSP4 transcript, a DNA fragment encoding part of the LDL receptor domain and the protease domain was used to probe an RNA blot composed of 76 different human tissues (catalog number 7775-1; human multiple tissue expression (MTE) array; CLONTECH). As in the northern analysis of gel blot, a very strong signal was observed in the liver. Signals in other tissues were observed in (decreasing signal level): fetal liver>heart=kidney=adrenal gland=testis=fetal heart and kidney=skeletal muscle=bladder=placenta>brain=spinal cord=colon=stomach=spleen=lymph node=bone marrow=trachea=uterus=pancreas=salivary gland=mammary gland=lung. MTSP4 is also expressed less abundantly in several tumor cell lines including HeLa S3=leukemia K-562=Burkitt's lymphomas (Raji and Daudi)=colorectal adenocarcinoma (SW480)>lung carcinoma (A549)=leukemia MOLT-4=leukemia HL-60. PCR of the MTSP4 transcript from cDNA libraries made from several human primary tumors xenografted in nude mice (human tumor multiple tissue cDNA panel, catalog number K1522-1, CLONTECH) was performed using MTSP4-specific primers. The MTSP4 transcript was detected in breast carcinoma (GI-101), lung carcinoma (LX-1), colon adenocarcinoma (GI-112) and pan-

creatic adenocarcinoma (GI-103). No apparent signal was detected in another form of lung carcinoma (GI-117), colon adenocarcinoma (CX-1), ovarian carcinoma (GI-102), and prostatic adenocarcinoma (PC3). The MTSP4 transcript was also detected in LNCaP and PC-3 prostate cancer cell lines as well as in HT-1080 human fibrosarcoma cell line.

[0272] Gene Expression Profile of MTSP6 in Normal and Tumor Tissues

[0273] To obtain information regarding the gene expression profile of the MTSP6 transcript, a 495 bp DNA fragment obtained from PCR reaction with primers Ch17-NSP-3 and NSP-4AS was used to probe an RNA blot composed of 76 different human tissues (catalog number 7775-1; human multiple tissue expression (MTE) array; CLONTECH). The strongest signal was observed in duodenum. Signal in other tissues were observed in (decreased signal level): Stomach>trachea=mammary gland=thyroid gland=salivary gland=pituitary gland=pancreas>kidney>lung>jejunum=ileum=ileocecum=appendix=fetal kidney>fetal lung. Very weak signals can also be detected in several other tissues.

[0274] MTSP6 is also expressed in several tumor cell lines including HeLa S3>colorectal adenocarcinoma (SW480)>leukemia MOLT-4>leukemia K-562. PCR analysis of the MTSP6 transcript from cDNA libraries made from several human primary tumors xenografted in nude mice (human tumor multiple tissue cDNA panel, catalog number K1522-1, CLONTECH) was performed using MTSP6-specific primers (Ch17-NSP-3 and Ch17-NSP2AS). The MTSP6 transcript was strongly detected in lung carcinoma (LX-1), moderately detected in pancreatic adenocarcinoma (GI-103), weakly detected in ovarian carcinoma (GI-102); and very weakly detected in colon adenocarcinoma (GI-112 and CX-1), breast carcinoma (GI-101), lung carcinoma (GI-117) and prostatic adenocarcinoma (PC3). The MTSP6 transcript was also detected in breast cancer cell line MDA-MB-231, prostate cancer cell line PC-3, but not in HT-1080 human fibrosarcoma cell line. MTSP6 is also expressed in mammary gland carcinoma cDNA (Clontech). MTSP6 is also over expressed in ovarian tumor cells.

[0275] D. Identification and Isolation of MTSP Protein Genes

[0276] The MTSP proteins, or domains thereof, can be obtained by methods well known in the art for protein purification and recombinant protein expression. Any method known to those of skill in the art for identification of nucleic acids that encode desired genes may be used. Any method available in the art can be used to obtain a full length (i.e., encompassing the entire coding region) cDNA or genomic DNA clone encoding an MTSP protein. In particular, the polymerase chain reaction (PCR) can be used to amplify a sequence identified as being differentially expressed in normal and tumor cells or tissues, e.g., nucleic acids encoding an MTSP protein (SEQ. NOS: 1-12), in a genomic or cDNA library. Oligonucleotide primers that hybridize to sequences at the 3' and 5' termini of the identified sequences can be used as primers to amplify by PCR sequences from a nucleic acid sample (RNA or DNA), preferably a cDNA library, from an appropriate source (e.g., tumor or cancer tissue).

[0277] PCR can be carried out, e.g., by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene

Amp™). The DNA being amplified can include mRNA or cDNA or genomic DNA from any eukaryotic species. One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to amplify nucleic acid homologs (e.g., to obtain MTSP protein sequences from species other than humans or to obtain human sequences with homology to MTSP protein) by allowing for greater or lesser degrees of nucleotide sequence similarity between the known nucleotide sequence and the nucleic acid homolog being isolated. For cross species hybridization, low stringency conditions are preferred. For same species hybridization, moderately stringent conditions are preferred. After successful amplification of the nucleic acid containing all or a portion of the identified MTSP protein sequence or of a nucleic acid encoding all or a portion of an MTSP protein homolog, that segment may be molecularly cloned and sequenced, and used as a probe to isolate a complete cDNA or genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis. Once the nucleotide sequence is determined, an open reading frame encoding the MTSP protein gene protein product can be determined by any method well known in the art for determining open reading frames, for example, using publicly available computer programs for nucleotide sequence analysis. Once an open reading frame is defined, it is routine to determine the amino acid sequence of the protein encoded by the open reading frame. In this way, the nucleotide sequences of the entire MTSP protein genes as well as the amino acid sequences of MTSP protein proteins and analogs may be identified.

[0278] Any eukaryotic cell potentially can serve as the nucleic acid source for the molecular cloning of the MTSP protein gene. The nucleic acids can be isolated from vertebrate, mammalian, human, porcine, bovine, feline, avian, equine, canine, as well as additional primate sources, insects, plants, etc. The DNA may be obtained by standard procedures known in the art from cloned DNA (e.g., a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (see, for example, Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Glover, D. M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K. Vol. I, II). Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

[0279] In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNase in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

[0280] Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene may be accomplished in a number of ways. For example, a portion of the MTSP protein (of any species) gene (e.g., a PCR amplification product obtained as described above or an oligonucleotide having a sequence of a portion of the known nucleotide sequence) or its specific RNA, or a fragment thereof be purified and labeled, and the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton and Davis, *Science* 196:180 (1977); Grunstein and Hogness, *Proc. Natl. Acad. Sci. U.S.A.* 72:3961 (1975)). Those DNA fragments with substantial homology to the probe will hybridize. It is also possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is available or by DNA sequence analysis and comparison to the known nucleotide sequence of MTSP protein. Further selection can be carried out on the basis of the properties of the gene. Alternatively, the presence of the gene may be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNA, can be selected which produce a protein that, e.g., has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion maps, antigenic properties, serine protease activity. If an anti-MTSP protein antibody is available, the protein may be identified by binding of labeled antibody to the putatively MTSP protein synthesizing clones, in an ELISA (enzyme-linked immunosorbent assay)-type procedure.

[0281] Alternatives to isolating the MTSP protein genomic DNA include, but are not limited to, chemically synthesizing the gene sequence from a known sequence or making cDNA to the mRNA that encodes the MTSP protein. For example, RNA for cDNA cloning of the MTSP protein gene can be isolated from cells expressing the protein. The identified and isolated nucleic acids can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene, La Jolla, Calif.). The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. If the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and MTSP protein gene may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

[0282] In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired

gene, for example, by size fractionization, can be done before insertion into the cloning vector.

[0283] In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated MTSP protein gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

[0284] E. Vectors, Plasmids and Cells that Contain Nucleic Acids Encoding an MTSP Protein or Protease Domain Thereof and Expression of MTSP Proteins

[0285] Vectors and Cells

[0286] For recombinant expression of one or more of the MTSP proteins, the nucleic acid containing all or a portion of the nucleotide sequence encoding the MTSP protein can be inserted into an appropriate expression vector, i.e., a vector that contains the necessary elements for the transcription and translation of the inserted protein coding sequence. The necessary transcriptional and translational signals can also be supplied by the native promoter for MTSP genes, and/or their flanking regions.

[0287] Also provided are vectors that contain nucleic acid encoding the MTSPs. Cells containing the vectors are also provided. The cells include eukaryotic and prokaryotic cells, and the vectors are any suitable for use therein.

[0288] Prokaryotic and eukaryotic cells, including endothelial cells, containing the vectors are provided. Such cells include bacterial cells, yeast cells, fungal cells, plant cells, insect cells and animal cells. The cells are used to produce an MTSP protein or protease domain thereof by growing the above-described cells under conditions whereby the encoded MTSP protein or protease domain of the MTSP protein is expressed by the cell, and recovering the expressed protease domain protein. For purposes herein, the protease domain is preferably secreted into the medium.

[0289] In one embodiment, the vectors include a sequence of nucleotides that encodes a polypeptide that has protease activity and contains all or a portion of only the protease domain, or multiple copies thereof, of an MTSP protein are provided. Also provided are vectors that comprise a sequence of nucleotides that encodes the protease domain and additional portions of an MTSP protein up to and including a full length MTSP protein, as well as multiple copies thereof, are also provided. The vectors may be selected for expression of the MTSP protein or protease domain thereof in the cell or such that the MTSP protein is expressed as a transmembrane protein. Alternatively, the vectors may include signals necessary for secretion of encoded proteins. When the protease domain is expressed the nucleic acid is preferably linked to a secretion signal, such as the *Saccharomyces cerevisiae*  $\alpha$  mating factor signal sequence or a portion thereof.

[0290] A variety of host-vector systems may be used to express the protein coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g. vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g. baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed

with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system used, any one of a number of suitable transcription and translation elements may be used.

[0291] Any methods known to those of skill in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene containing of appropriate transcriptional/translational control signals and protein coding sequences. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinants (genetic recombination). Expression of nucleic acid sequences encoding MTSP protein, or domains, derivatives, fragments or homologs thereof, may be regulated by a second nucleic acid sequence so that the genes or fragments thereof are expressed in a host transformed with the recombinant DNA molecule(s). For example, expression of the proteins may be controlled by any promoter/enhancer known in the art. In a specific embodiment, the promoter is not native to the genes for MTSP protein. Promoters which may be used include but are not limited to the SV40 early promoter (Bernoist and Chambon, *Nature* 290:304-310 (1981)), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980)), the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. USA* 78:1441-1445 (1981)), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39-42 (1982)); prokaryotic expression vectors such as the  $\beta$ -lactamase promoter (Villa-Kamaroff et al., *Proc. Natl. Acad. Sci. USA* 75:3727-3731 (1978)) or the tac promoter (DeBoer et al., *Proc. Natl. Acad. Sci. USA* 80:21-25 (1983)); see also "Useful Proteins from Recombinant Bacteria": in *Scientific American* 242:79-94 (1980)); plant expression vectors containing the nopaline synthetase promoter (Herrar-Estrella et al., *Nature* 303:209-213 (1984)) or the cauliflower mosaic virus 35S RNA promoter (Gardner et al., *Nucleic Acids Res.* 9:2871 (1981)), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., *Nature* 310:115-120 (1984)); promoter elements from yeast and other fungi such as the Gal4 promoter, the alcohol dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline phosphatase promoter, and the following animal transcriptional control regions that exhibit tissue specificity and have been used in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., *Cell* 38:639-646 (1984); Ornitz et al., *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409 (1986); MacDonald, *Hepatology* 7:425-515 (1987)); insulin gene control region which is active in pancreatic beta cells (Hanahan et al., *Nature* 315:115-122 (1985)), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., *Cell* 38:647-658 (1984); Adams et al., *Nature* 318:533-538 (1985); Alexander et al., *Mol. Cell Biol.* 7:1436-1444 (1987)), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., *Cell* 45:485-495 (1986)), albumin gene control region which is active in liver (Pinckert et al., *Genes and Devel.* 1:268-276 (1987)), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., *Mol. Cell. Biol.* 5:1639-1648 (1985); Hammer et al., *Science* 235:53-58 (1987)), alpha-1 antitrypsin gene control region which is active in liver (Kelsey et al., *Genes and Devel.* 1:161-171 (1987)), beta globin gene control region which is

active in myeloid cells (Mogram et al., *Nature* 315:338-340 (1985); Kollias et al., *Cell* 46:89-94 (1986)), myelin basic protein gene control region which is active in oligodendrocyte cells of the brain (Readhead et al., *Cell* 48:703-712 (1987)), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, *Nature* 314:283-286 (1985)), and gonadotrophic releasing hormone gene control region which is active in gonadotrophs of the hypothalamus (Mason et al., *Science* 234:1372-1378 (1986)).

[0292] In a specific embodiment, a vector is used that contains a promoter operably linked to nucleic acids encoding an MTSP protein, or a domain, fragment, derivative or homolog, thereof, one or more origins of replication, and optionally, one or more selectable markers (e.g., an antibiotic resistance gene). Expression vectors containing the coding sequences, or portions thereof, of an MTSP protein, is made, for example, by subcloning the coding portions into the EcoRI restriction site of each of the three pGEX vectors (glutathione S-transferase expression vectors (Smith and Johnson, *Gene* 7:31-40 (1988)). This allows for the expression of products in the correct reading frame. Preferred vectors and systems for expression of the protease domains of the MTSP proteins are well known Pichia vectors (available, for example, from Invitrogen, San Diego, Calif.), particularly those designed for secretion of the encoded proteins. One exemplary vector is described in the EXAMPLES.

[0293] Plasmids for transformation of *E. coli* cells, include, for example, the pET expression vectors (see, U.S. Pat. No. 4,952,496; available from NOVAGEN, Madison, Wis.; see, also literature published by Novagen describing the system). Such plasmids include pET 11a, which contains the T7lac promoter, T7 terminator, the inducible *E. coli* lac operator, and the lac repressor gene; pET 12a-c, which contains the T7 promoter, T7 terminator, and the *E. coli* ompT secretion signal; and pET 15b and pET19b (NOVAGEN, Madison, Wis.), which contain a His-Tag™ leader sequence for use in purification with a His column and a thrombin cleavage site that permits cleavage following purification over the column; the T7-lac promoter region and the T7 terminator.

[0294] The vectors are introduced into host cells, such as Pichia cells and bacterial cells, such as *E. coli*, and the proteins expressed therein. Preferred Pichia strains, include, for example, GS115. Preferred bacterial hosts contain chromosomal copies of DNA encoding T7 RNA polymerase operably linked to an inducible promoter, such as the lacUV promoter (see, U.S. Pat. No. 4,952,496). Such hosts include, but are not limited to, the lysogenic *E. coli* strain BL21 (DE3).

[0295] Expression and Production of Proteins

[0296] The MTSP domains, derivatives and analogs be produced by various methods known in the art. For example, once a recombinant cell expressing an MTSP protein, or a domain, fragment or derivative thereof, is identified, the individual gene product can be isolated and analyzed. This is achieved by assays based on the physical and/or functional properties of the protein, including, but not limited to, radioactive labeling of the product followed by analysis by gel electrophoresis, immunoassay, cross-linking to marker-labeled product. The MTSP protein proteins may be isolated and purified by standard methods known in the art (either

from natural sources or recombinant host cells expressing the complexes or proteins), including but not restricted to column chromatography (e.g., ion exchange, affinity, gel exclusion, reversed-phase high pressure, fast protein liquid, etc.), differential centrifugation, differential solubility, or by any other standard technique used for the purification of proteins. Functional properties may be evaluated using any suitable assay known in the art.

[0297] Alternatively, once an MTSP protein or its domain or derivative is identified, the amino acid sequence of the protein can be deduced from the nucleotide sequence of the gene which encodes it. As a result, the protein or its domain or derivative can be synthesized by standard chemical methods known in the art (e.g. see Hunkapiller et al, *Nature* 310:105-111 (1984)).

[0298] Manipulations of MTSP protein sequences may be made at the protein level. Also contemplated herein are MTSP protein proteins, domains thereof, derivatives or analogs or fragments thereof, which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH<sub>4</sub>, acetylation, formulation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

[0299] In addition, domains, analogs and derivatives of an MTSP protein can be chemically synthesized. For example, a peptide corresponding to a portion of an MTSP protein, which includes the desired domain or which mediates the desired activity in vitro can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the MTSP protein sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-aminobutyric acid, ε-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0300] In cases where natural products are suspected of being mutant or are isolated from new species, the amino acid sequence of the MTSP protein isolated from the natural source, as well as those expressed in vitro, or from synthesized expression vectors in vivo or in vitro, can be determined from analysis of the DNA sequence, or alternatively, by direct sequencing of the isolated protein. Such analysis may be performed by manual sequencing or through use of an automated amino acid sequenator.

[0301] Modifications

[0302] A variety of modification of the MTSP proteins and domains are contemplated herein. An MTSP-encoding

nucleic acid molecule be modified by any of numerous strategies known in the art (Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). The sequences can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated in vitro. In the production of the gene encoding a domain, derivative or analog of MTSP, care should be taken to ensure that the modified gene retains the original translational reading frame, uninterrupted by translational stop signals, in the gene region where the desired activity is encoded.

[0303] Additionally, the MTSP-encoding nucleic acid molecules can be mutated in vitro or in vivo, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further in vitro modification. Also, as described herein muteins with primary sequence alterations, such as replacements of Cys residues and elimination of glycosylation sites are contemplated. Such mutations may be effected by any technique for mutagenesis known in the art, including, but not limited to, chemical mutagenesis and in vitro site-directed mutagenesis (Hutchinson et al., *J. Biol. Chem.* 253:6551-6558 (1978)), use of TAB® linkers (Pharmacia). In one embodiment, for example, an MTSP protein or domain thereof is modified to include a fluorescent label. In other specific embodiments, the MTSP protein is modified to have a heterofunctional reagent, such heterofunctional reagents can be used to crosslink the members of the complex.

[0304] The MTSP proteins may be isolated and purified by standard methods known in the art (either from natural sources or recombinant host cells expressing the complexes or proteins), including but not restricted to column chromatography (e.g., ion exchange, affinity, gel exclusion, reversed-phase high pressure, fast protein liquid, etc.), differential centrifugation, differential solubility, or by any other standard technique used for the purification of proteins. Functional properties may be evaluated using any suitable assay known in the art.

[0305] Alternatively, once a MTSP or its domain or derivative is identified, the amino acid sequence of the protein can be deduced from the nucleotide sequence of the gene which encodes it. As a result, the protein or its domain or derivative can be synthesized by standard chemical methods known in the art (e.g., see Hunkapiller et al, *Nature*, 310:105-111 (1984)).

[0306] Manipulations of MTSP sequences may be made at the protein level. MTSP domains, derivatives or analogs or fragments, which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule and other cellular ligand, are contemplated herein. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH<sub>4</sub>, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

[0307] In addition, domains, analogs and derivatives of a MTSP can be chemically synthesized. For example, a pep-

ptide corresponding to a portion of a MTSP, which comprises the desired domain or which mediates the desired activity in vitro can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the MTSP sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-aminobutyric acid,  $\epsilon$ -Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteine acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids such as  $\beta$ -methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

#### [0308] F. Screening Methods

[0309] The single chain protease domains, as shown herein, can be used in a variety of methods to identify compounds that modulate the activity thereof. For MTSPs that exhibit higher activity or expression in tumor cells, compounds that inhibit the proteolytic activity are of particular interest. For any MTSPs that are active at lower levels in tumor cells, compounds or agents that enhance the activity are potentially of interest. In all instances the identified compounds will include agents that are candidate cancer treatments.

[0310] Several types of assays are exemplified and described herein. It is understood that the protease domains may be used in other assays. It is shown here, however, that the single chain protease domains exhibit catalytic activity. As such they are ideal for in vitro screening assays. They may also be used in binding assays.

[0311] The MTSP3, MTSP4 and MTSP6 full length zymogens, activated enzymes, single and double chain protease domains are contemplated for use in any screening assay known to those of skill in the art, including those provided herein. Hence the following description, if directed to proteolytic assays is intended to apply to use of a single chain protease domain or a catalytically active portion thereof of any MTSP, including an MTSP3, MTSP4 or an MTSP6. Other assays, such as binding assays are provided herein, particularly for use with an MTSP3, MTSP4 or MTSP6, including any variants, such as splice variants thereof. MTSP3 and MTSP4 are of most interest in such assays.

#### [0312] 1. Catalytic Assays for Identification of Agents that Modulate the Protease Activity of an MTSP Protein

[0313] Methods for identifying a modulator of the catalytic activity of an MTSP, particularly a single chain protease domain or catalytically active portion thereof, are provided herein. The methods can be practiced by: a) contacting the MTSP, particularly a single-chain domain thereof, with a substrate of the MTSP in the presence of a test substance, and detecting the proteolysis of the substrate, whereby the activity of the MTSP is assessed, and comparing the activity to a control. For example, the control can be the activity of the MTSP assessed by contacting an MTSP, particularly a single-chain domain thereof, with a substrate of the MTSP, and detecting the proteolysis of the substrate,

whereby the activity of the MTSP is assessed. The results in the presence and absence of the test compounds are compared. A difference in the activity indicates that the test substance modulates the activity of the MTSP.

[0314] In one embodiment a plurality of the test substances are screened simultaneously in the above screening method. In another embodiment, the MTSP is isolated from a target cell as a means for then identifying agents that are potentially specific for the target cell.

[0315] In still another embodiment, The test substance is a therapeutic compound, and whereby a difference of the MTSP activity measured in the presence and in the absence of the test substance indicates that the target cell responds to the therapeutic compound.

[0316] One method include the steps of (a) contacting the MTSP protein or protease domain thereof with one or a plurality of test compounds under conditions conducive to interaction between the ligand and the compounds; and (b) identifying one or more compounds in the plurality that specifically binds to the ligand.

[0317] Another method provided herein includes the steps of a) contacting an MTSP protein or protease domain thereof with a substrate of the MTSP protein, and detecting the proteolysis of the substrate, whereby the activity of the MTSP protein is assessed; b) contacting the MTSP protein with a substrate of the MTSP protein in the presence of a test substance, and detecting the proteolysis of the substrate, whereby the activity of the MTSP protein is assessed; and c) comparing the activity of the MTSP protein assessed in steps a) and b), whereby the activity measured in step a) differs from the activity measured in step b) indicates that the test substance modulates the activity of the MTSP protein.

[0318] In another embodiment, a plurality of the test substances are screened simultaneously. In comparing the activity of an MTSP protein in the presence and absence of a test substance to assess whether the test substance is a modulator of the MTSP protein, it is unnecessary to assay the activity in parallel, although such parallel measurement is preferred. It is possible to measure the activity of the MTSP protein at one time point and compare the measured activity to a historical value of the activity of the MTSP protein.

[0319] For instance, one can measure the activity of the MTSP protein in the presence of a test substance and compare with historical value of the activity of the MTSP protein measured previously in the absence of the test substance, and vice versa. This can be accomplished, for example, by providing the activity of the MTSP protein on an insert or pamphlet provided with a kit for conducting the assay.

[0320] Methods for selecting substrates for a particular MTSP are described in the EXAMPLES, and particular proteolytic assays are exemplified.

[0321] Combinations and kits containing the combinations optionally including instructions for performing the assays are provided. The combinations include an MTSP protein and a substrate of the MTSP protein to be assayed; and, optionally reagents for detecting proteolysis of the substrate. The substrates, which are typically proteins subject to proteolysis by a particular MTSP protein, can be identified

empirically by testing the ability of the MTSP protein to cleave the test substrate. Substrates that are cleaved most effectively (i.e., at the lowest concentrations and/or fastest rate or under desirable conditions), are identified.

[0322] Additionally provided herein is a kit containing the above-described combination. Preferably, the kit further includes instructions for identifying a modulator of the activity of an MTSP protein. Any MTSP protein is contemplated as target for identifying modulators of the activity thereof.

#### [0323] 2. Binding Assays

[0324] Also provided herein are methods for identification and isolation of agents, particularly compounds that bind to MTSPs. The assays are designed to identify agents that bind to the zymogen form, the single chain isolated protease domain (or a protein, other than an MTSP protein, that contains the protease domain of an MTSP protein), and to the activated form, including the activated form derived from the full length zymogen or from an extended protease domain. The identified compounds are candidates or leads for identification of compounds for treatments of tumors and other disorders and diseases involving aberrant angiogenesis. The MTSP proteins used in the methods include any MTSP protein as defined herein, and preferably use MTSP single chain domain or proteolytically active portion thereof.

[0325] A variety of methods are provided herein. These methods may be performed in solution or in solid phase reactions in which the MTSP protein(s) or protease domain(s) thereof are linked, either directly or indirectly via a linker, to a solid support. Screening assays are described in the Examples, and these assays have been used to identify candidate compounds.

[0326] For purposes herein, all binding assays described above are provided for MTSP3, MTSP4 and MTSP6 (CHECK REF). For MTSP1 (including any variant thereof) and other such proteases, binding assays that employ the isolated single chain protease domain or a protein containing such domain (other than the MTSP from which the protease is derived) are provided.

[0327] Methods for identifying an agent, such as a compound, that specifically binds to an MTSP single chain protease domain or an MTSP, such as an MTSP3, MTSP4 or an MTSP6, are provided herein. The method can be practiced by (a) contacting the MTSP with one or a plurality of test agents under conditions conducive to binding between the MTSP and an agent; and (b) identifying one or more agents within the plurality that specifically binds to the MTSP.

[0328] For example, in practicing such methods the MTSP polypeptide is mixed with a potential binding partner or an extract or fraction of a cell under conditions that allow the association of potential binding partners with the polypeptide. After mixing, peptides, polypeptides, proteins or other molecules that have become associated with an MTSP are separated from the mixture. The binding partner that bound to the MTSP can then be removed and further analyzed. To identify and isolate a binding partner, the entire protein, for instance the entire disclosed protein of SEQ ID Nos. 6, 8 10 or 12 can be used. Alternatively, a fragment of the protein can be used.

[0329] A variety of methods can be used to obtain cell extracts. Cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods include, but are not limited to, detergent lysis and enzyme lysis. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain extracts for use in the present methods.

[0330] Once an extract of a cell is prepared, the extract is mixed with the MTSP under conditions in which association of the protein with the binding partner can occur. A variety of conditions can be used, the most preferred being conditions that closely resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH, temperature, and the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner.

[0331] After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be used to separate the mixture. For example, antibodies specific to an MTSP can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density/sediment centrifugation can be used.

[0332] After removing the non-associated cellular constituents in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

[0333] To aid in separating associated binding partner pairs from the mixed extract, the MTSP can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads.

[0334] Attachment of the protein or a fragment thereof to a solid support aids in separating peptide/binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of two or more proteins.

[0335] Alternatively, the nucleic acid molecules encoding the single chain proteases can be used in a yeast two-hybrid system. The yeast two-hybrid system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described.

[0336] Another in vitro binding assay, particularly for an MTSP3, MTSP4 or an MTSP6 uses a mixture of a polypeptide that contains at least the catalytic domain of one of these proteins and one or more candidate binding targets or substrates. After incubating the mixture under appropriate conditions, one determines whether the MTSP or a polypeptide fragment thereof containing the catalytic domain binds with the candidate substrate. For cell-free binding assays, one of the components includes or is coupled to a detectable label. The label may provide for direct detection, such as radioactivity, luminescence, optical or electron density, etc., or indirect detection such as an epitope tag, an enzyme, etc. A variety of methods may be employed to detect the label depending on the nature of the label and other assay components. For example, the label may be detected bound to the solid substrate or a portion of the bound complex

containing the label may be separated from the solid substrate, and the label thereafter detected.

**[0337]** 3. Detection of Signal Transduction

**[0338]** The cell surface location of the MTSPs suggests a role for some or all of these proteins in signal transduction. Assays for assessing signal transduction are well known to those of skill in the art, and may be adapted for use with the MTSP protein.

**[0339]** Assays for identifying agents that effect or alter signal transduction mediated by an MTSP, particularly the full length or a sufficient portion to anchor the extracellular domain or a function portion thereof of an MTSP on the surface of a cell are provided. Such assays, include, for example, transcription based assays in which modulation of a transduced signal is assessed by detecting an effect on an expression from a reporter gene (see, e.g., U.S. Pat. No. 5,436,128).

**[0340]** 4. Methods for Identifying Agents that Modulate the Expression a Nucleic Acid Encoding an MTSP, particularly an MTSP3, MTSP4 or MTSP6

**[0341]** Another embodiment provides methods for identifying agents that modulate the expression of a nucleic acid encoding an MTSP, particularly an MTSP3, MTSP4 or MTSP6. Such assays use any available means of monitoring for changes in the expression level of the nucleic acids encoding an MTSP, such as MTSP3 or MTSP4.

**[0342]** In one assay format, cell lines that contain reporter gene fusions between the open reading frame of MTSP3, MTSP4 or MTSP6 or a domain thereof, particularly the protease domain and any assayable fusion partner may be prepared. Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam et al., *Anal. Biochem.* 188: 245-54 (1990)). Cell lines containing the reporter gene fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid encoding an MTSP3, MTSP4 or MTSP6.

**[0343]** Additional assay formats may be used to monitor the ability of the agent to modulate the expression of a nucleic acid encoding an MTSP3, MTSP4 or MTSP6. For instance, mRNA expression may be monitored directly by hybridization to the nucleic acids. Cell lines are exposed to the agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures (see, e.g., Sambrook et al. (1989) *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed. Cold Spring Harbor Laboratory Press). Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells may be prepared from the nucleic acids. It is preferable, but not necessary, to design probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid hybrids form under conditions of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between two nucleic acid strands in order to form a hybrid. Stringency should be chosen to maximize the difference in stability between the probe:target hybrid and potential probe:non-target hybrids.

**[0344]** Probes may be designed from the nucleic acids through methods known in the art. For instance, the G+C content of the probe and the probe length can affect probe binding to its target sequence. Methods to optimize probe specificity are commonly available (see, e.g., Sambrook et al. (1989) *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed. Cold Spring Harbor Laboratory Press); and Ausubel et al. (1995) *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, Greene Publishing Co., NY).

**[0345]** Hybridization conditions are modified using known methods (see, e.g., Sambrook et al. (1989) *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed. Cold Spring Harbor Laboratory Press); and Ausubel et al. (1995) *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, Greene Publishing Co., NY), as required for each probe. Hybridization of total cellular RNA or RNA enriched for polyA RNA can be accomplished in any available format. For instance, total cellular RNA or RNA enriched for polyA RNA can be affixed to a solid support, and the solid support exposed to at least one probe comprising at least one, or part of one of the nucleic acid molecules under conditions in which the probe will specifically hybridize. Alternatively, nucleic acid fragments comprising at least one, or part of one of the sequences can be affixed to a solid support, such as a porous glass wafer. The glass wafer can then be exposed to total cellular RNA or polyA RNA from a sample under conditions in which the affixed sequences will specifically hybridize. Such glass wafers and hybridization methods are widely available, for example, those disclosed by Beattie (WO 95/11755). By examining for the ability of a given probe to specifically hybridize to an RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up or down regulate the expression of a nucleic acid encoding the protein having the sequence of SEQ ID NO:3 or SEQ ID NO:4 are identified.

**[0346]** 5. Methods for Identifying Agents that Modulate at Least One Activity of an MTSP, such as MTSP3, MTSP4 or MTSP6

**[0347]** Methods for identifying agents that modulate at least one activity of an MTSP, such as an MTSP3, MTSP4 or MTSP6 are provided. Such methods or assays may use any means of monitoring or detecting the desired activity.

**[0348]** In one format, the relative amounts of a protein between a cell population that has been exposed to the agent to be tested compared to an un-exposed control cell population may be assayed (e.g., a prostate cancer cell line, a lung cancer cell line, a colon cancer cell line or a breast cancer cell line). In this format, probes, such as specific antibodies, are used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates may be prepared from the exposed cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe.

**[0349]** For example, N- and C- terminal fragments of the MTSP can be expressed in bacteria and used to search for proteins which bind to these fragments. Fusion proteins, such as His-tag or GST fusion to the N- or C-terminal regions of the MTSP, such as an MTSP3, MTSP4 or an MTSP6, can be prepared for use as a substrate. These fusion

proteins can be coupled to, for example, Glutathione-Sepharose beads and then probed with cell lysates. Prior to lysis, the cells may be treated with a candidate agent which may modulate an MTSP, such as an MTSP3, MTSP4 or an MTSP6, or proteins that interact with domains thereon. Lysate proteins binding to the fusion proteins can be resolved by SDS-PAGE, isolated and identified by protein sequencing or mass spectroscopy, as is known in the art.

[0350] Antibody probes are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the peptides, polypeptides or proteins if they are of sufficient length (e.g., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40 or more consecutive amino acids) the MTSP protein, such as an MTSP3, an MTSP4 or an MTSP6), or if required to enhance immunogenicity, conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers, such as bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, Ill., may be desirable to provide accessibility to the hapten. Hapten peptides can be extended at either the amino or carboxy terminus with a Cys residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

[0351] Anti-peptide antibodies can be generated using synthetic peptides corresponding to, for example, the carboxy terminal amino acids of the MTSP. Synthetic peptides can be as small as 1-3 amino acids in length, but are preferably at least 4 or more amino acid residues long. The peptides can be coupled to KLH using standard methods and can be immunized into animals, such as rabbits or ungulate. Polyclonal antibodies can then be purified, for example using Actigel beads containing the covalently bound peptide.

[0352] While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler et al., (*Nature* 256: 495-7 (1975)) or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten, polypeptide or protein. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either in vitro or by production in vivo via ascites fluid. Of particular interest, are monoclonal antibodies that recognize the catalytic domain of the an MTSP, such as an MTSP3, MTSP4 or an MTSP6.

[0353] Additionally, the zymogen or two-chain forms of the MTSP can be used to make monoclonal antibodies which recognize conformation epitopes. For peptide-directed monoclonal antibodies, peptides from the C1r/C1s domain can be used to generate anti-C1r/C1s domain monoclonal

antibodies which can thereby block activation of the zymogen to the two-chain form of the MTSP. This domain can similarly be the substrate for other non-antibody compounds which bind to these preferred domains on either the single-chain or double-chain forms of the MTSP3, MTSP4 or MTSP6, and thereby modulate the activity of thereof or prevent its activation.

[0354] The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', or F(ab')<sub>2</sub> fragments are often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

[0355] The antibodies or fragments may also be produced. Regions that bind specifically to the desired regions of receptor can also be produced in the context of chimeras with multiple species origin.

[0356] Agents that are assayed in the above method can be randomly selected or rationally selected or designed.

[0357] The agents can be, as examples, peptides, small molecules, and carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents.

[0358] The peptide agents can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

[0359] G. Assay Formats and Selection of Test Substances

[0360] A variety of formats and detection protocols are known for performing screening assays. Any such formats and protocols may be adapted for identifying modulators of MTSP protein activities. The following includes a discussion of exemplary protocols.

[0361] 1. High Throughput Screening Assays

[0362] Although the above-described assay can be conducted where a single MTSP protein is screened, and/or a single test substance is screened for in one assay, the assay is preferably conducted in a high throughput screening mode, i.e., a plurality of the MTSP proteins are screened against and/or a plurality of the test substances are screened for simultaneously (See generally, *High Throughput Screening: The Discovery of Bioactive Substances* (Devlin, Ed.) Marcel Dekker, 1997; Sittampalam et al., *Curr. Opin. Chem. Biol.*, 1(3):384-91 (1997); and Silverman et al., *Curr. Opin. Chem. Biol.*, 2(3):397-403 (1998)). For example, the assay can be conducted in a multi-well (e.g., 24-, 48-, 96-, or 384-well), chip or array format.

[0363] High-throughput screening (HTS) is the process of testing a large number of diverse chemical structures against disease targets to identify "hits" (Sittampalam et al., *Curr.*

*Opin. Chem. Biol.*, 1(3):384-91 (1997)). Current state-of-the-art HTS operations are highly automated and computerized to handle sample preparation, assay procedures and the subsequent processing of large volumes of data.

[0364] Detection technologies employed in high-throughput screens depend on the type of biochemical pathway being investigated (Sittampalam et al., *Curr. Opin. Chem. Biol.*, 1(3):384-91 (1997)). These methods include, radiochemical methods, such as the scintillation proximity assays (SPA), which can be adapted to a variety of enzyme assays (Lerner et al., *J. Biomol. Screening*, 1:135-143 (1996); Baker et al., *Anal. Biochem.*, 239:20-24 (1996); Baum et al., *Anal. Biochem.*, 237:129-134 (1996); and Sullivan et al., *J. Biomol. Screening*, 2:19-23 (1997)) and protein-protein interaction assays (Braunwalder et al., *J. Biomol. Screening*, 1:23-26 (1996); Sonatore et al., *Anal. Biochem.*, 240:289-297 (1996); and Chen et al., *J. Biol. Chem.*, 271:25308-25315 (1996)), and non-isotopic detection methods, including but are not limited to, calorimetric and luminescence detection methods, resonance energy transfer (RET) methods, time-resolved fluorescence (HTRF) methods, cell-based fluorescence assays, such as fluorescence resonance energy transfer (FRET) procedures (see, e.g., Gonzalez et al., *Biophys. J.*, 69:1272-1280 (1995)), fluorescence polarization or anisotropy methods (see, e.g., Jameson et al., *Methods Enzymol.*, 246:283-300 (1995); Jolley, *J. Biomol. Screening*, 1:33-38 (1996); Lynch et al., *Anal. Biochem.*, 247:77-82 (1997)), fluorescence correlation spectroscopy (FCS) and other such methods.

#### [0365] 2. Test Substances

[0366] Test compounds, including small molecules and libraries and collections thereof can be screened in the above-described assays and assays described below to identify compounds that modulate the activity of an MTSP protein. Rational drug design methodologies that rely on computational chemistry may be used to screen and identify candidate compounds.

[0367] The compounds identified by the screening methods include inhibitors, including antagonists, and may be agonists. Compounds for screening are any compounds and collections of compounds available, known or that can be prepared.

#### [0368] a. Selection of Compounds

[0369] Compounds can be selected for their potency and selectivity of inhibition of serine proteases, especially MTSP protein. As described herein, and as generally known, a target serine protease and its substrate are combined under assay conditions permitting reaction of the protease with its substrate. The assay is performed in the absence of test compound, and in the presence of increasing concentrations of the test compound. The concentration of test compound at which 50% of the serine protease activity is inhibited by the test compound is the IC<sub>50</sub> value (Inhibitory Concentration) or EC<sub>50</sub> (Effective Concentration) value for that compound. Within a series or group of test compounds, those having lower IC<sub>50</sub> or EC<sub>50</sub> values are considered more potent inhibitors of the serine protease than those compounds having higher IC<sub>50</sub> or EC<sub>50</sub> values. The IC<sub>50</sub> measurement is often used for more simplistic assays, whereas the EC<sub>50</sub> is often used for more complicated assays, such as those employing cells.

[0370] Preferred compounds according to this aspect have an IC<sub>50</sub> value of 100 nM or less as measured in an in vitro assay for inhibition of MTSP protein activity. Especially preferred compounds have an IC<sub>50</sub> value of less than 100 nM.

[0371] The test compounds also are evaluated for selectivity toward a serine protease. As described herein, and as generally known, a test compound is assayed for its potency toward a panel of serine proteases and other enzymes and an IC<sub>50</sub> value or EC<sub>50</sub> value is determined for each test compound in each assay system. A compound that demonstrates a low IC<sub>50</sub> value or EC<sub>50</sub> value for the target enzyme, e.g., MTSP protein, and a higher IC<sub>50</sub> value or EC<sub>50</sub> value for other enzymes within the test panel (e.g., urokinase tissue plasminogen activator, thrombin, Factor Xa), is considered to be selective toward the target enzyme. Generally, a compound is deemed selective if its IC<sub>50</sub> value or EC<sub>50</sub> value in the target enzyme assay is at least one order of magnitude less than the next smallest IC<sub>50</sub> value or EC<sub>50</sub> value measured in the selectivity panel of enzymes.

[0372] Presently preferred compounds have an IC<sub>50</sub> value of 100 nM or less as measured in an in vitro assay for inhibition of urokinase activity. Especially preferred compounds have an IC<sub>50</sub> value in the in vitro urokinase inhibition assay that is at least one order of magnitude smaller than the IC<sub>50</sub> value measured in the in vitro tPA inhibition assay. Compounds having a selectivity ratio of IC<sub>50</sub> u-PA assay: IC<sub>50</sub> MTSP protein assay of greater than 100 are especially preferred.

[0373] Compounds are also evaluated for their activity in vivo. The type of assay chosen for evaluation of test compounds will depend on the pathological condition to be treated or prevented by use of the compound, as well as the route of administration to be evaluated for the test compound.

[0374] For instance, to evaluate the activity of a compound to reduce tumor growth through inhibition of MTSP protein, the procedures described by Jankun et al., *Canc. Res.*, 57:559-563 (1997) to evaluate PAI-1 can be employed. Briefly, the ATCC cell lines DU145 and LnCaP are injected into SCID mice. After tumors are established, the mice are given test compound according to a dosing regime determined from the compound's in vitro characteristics. The Jankun et al. compound was administered in water. Tumor volume measurements are taken twice a week for about five weeks. A compound is deemed active if an animal to which the compound was administered exhibited decreased tumor volume, as compared to animals receiving appropriate control compounds.

[0375] Another in vivo experimental model designed to evaluate the effect of p-aminobenzamidine, a swine protease inhibitor, on reducing tumor volume is described by Billstrom et al., *Int. J. Cancer*, 61:542-547 (1995).

[0376] To evaluate the ability of a compound to reduce the occurrence of, or inhibit, metastasis, the procedures described by Kobayashi et al., *Int. J. Canc.*, 57:727-733d (1994) can be employed. Briefly, a murein xenograft selected for high lung colonization potential is injected into C57B1/6 mice i.v. (experimental metastasis) or s.c. into the abdominal wall (spontaneous metastasis). Various concentrations of the compound to be tested can be admixed with

the tumor cells in Matrigel prior to injection. Daily i.p. injections of the test compound are made either on days 1-6 or days 7-13 after tumor inoculation. The animals are sacrificed about three or four weeks after tumor inoculation, and the lung tumor colonies are counted. Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing and route of administration.

[0377] The activity of the tested compounds toward decreasing tumor volume and metastasis can be evaluated in model described in Rabbani et al., *Int. J. Cancer* 63:840-845 (1995) to evaluate their inhibitor. There, Mat LyLu tumor cells were injected into the flank of Copenhagen rats. The animals were implanted with osmotic minipumps to continuously administer various doses of test compound for up to three weeks. The tumor mass and volume of experimental and control animals were evaluated during the experiment, as were metastatic growths. Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing, and route of administration. Some of these authors described a related protocol in Xing et al., *Canc. Res.*, 57:3585-3593 (1997).

[0378] To evaluate the inhibitory activity of a compound, a rabbit cornea neovascularization model can be employed. Avery et al., *Arch. Ophthalmol.*, 108:1474-1475 (1990) describe anesthetizing New Zealand albino rabbits and then making a central corneal incision and forming a radial corneal pocket. A slow release prostaglandin pellet was placed in the pocket to induce neovascularization. Test compound was administered i.p. for five days, at which time the animals were sacrificed. The effect of the test compound is evaluated by review of periodic photographs taken of the limbus, which can be used to calculate the area of neovascular response and, therefore, limbal neovascularization. A decreased area of neovascularization as compared with appropriate controls indicates the test compound was effective at decreasing or inhibiting neovascularization.

[0379] As angiogenesis model used to evaluate the effect of a test compound in preventing angiogenesis is described by Min et al., *Canc. Res.*, 56:2428-2433 (1996). C57BL6 mice receive subcutaneous injections of a Matrigel mixture containing bFGF, as the angiogenesis-inducing agent, with and without the test compound. After five days, the animals are sacrificed and the Matrigel plugs, in which neovascularization can be visualized, are photographed. An experimental animal receiving Matrigel and an effective dose of test compound will exhibit less vascularization than a control animal or an experimental animal receiving a less- or non-effective dose of compound.

[0380] An in vivo system designed to test compounds for their ability to limit the spread of primary tumors is described by Crowley et al., *Proc. Natl. Acad. Sci.*, 90:5021-5025 (1993). Nude mice are injected with tumor cells (PC3) engineered to express CAT (chloramphenicol acetyltransferase). Compounds to be tested for their ability to decrease tumor size and/or metastases are administered to the animals, and subsequent measurements of tumor size and/or metastatic growths are made. In addition, the level of CAT detected in various organs provides an indication of the ability of the test compound to inhibit metastasis; detection of less CAT in tissues of a treated animal versus a control animal indicates less CAT-expressing cells migrated to that tissue.

[0381] In vivo experimental modes designed to evaluate the inhibitory potential of a test serine protease inhibitors, using a tumor cell line F3II, the to be highly invasive, are described by Alonso et al., *Breast Canc. Res. Treat.*, 40:209-223 (1996). This group describes in vivo studies for toxicity determination, tumor growth, invasiveness, spontaneous metastasis, experimental lung metastasis, and an angiogenesis assay.

[0382] The CAM model (chick embryo chorioallantoic membrane model), first described by L. Ossowski in 1998 (*J. Cell Biol.*, 107:2437-2445 (1988)), provides another method for evaluating the urokinase inhibitory activity of a test compound. In the CAM model, tumor cells invade through the chorioallantoic membrane containing CAM with tumor cells in the presence of several serine protease inhibitors results in less or no invasion of the tumor cells through the membrane. Thus, the CAM assay is performed with CAM and tumor cells in the presence and absence of various concentrations of test compound. The invasiveness of tumor cells is measured under such conditions to provide an indication of the compound's inhibitory activity. A compound having inhibitory activity correlates with less tumor invasion.

[0383] The CAM model is also used in a standard assay of angiogenesis (i.e., effect on formation of new blood vessels (Brooks et al., *Methods in Molecular Biology*, 129:257-269 (1999)). According to this model, a filter disc containing an angiogenesis inducer, such as basic fibroblast growth factor (bFGF) is placed onto the CAM. Diffusion of the cytokine into the CAM induces local angiogenesis, which may be measured in several ways such as by counting the number of blood vessel branch points within the CAM directly below the filter disc. The ability of identified compounds to inhibit cytokine-induced angiogenesis can be tested using this model. A test compound can either be added to the filter disc that contains the angiogenesis inducer, be placed directly on the membrane or be administered systemically. The extent of new blood vessel formation in the presence and/or absence of test compound can be compared using this model. The formation of fewer new blood vessels in the presence of a test compound would be indicative of anti-angiogenesis activity. Demonstration of anti-angiogenesis activity for inhibitors of an MTSP protein indicates a role in angiogenesis for that MTSP protein.

#### [0384] b. Known Serine Protease Inhibitors

[0385] Compounds for screening can be serine protease inhibitors, which can be tested for their ability to inhibit the activity of an MTSP, particularly an MTSP3, MTSP4, or MTSP6.

[0386] Exemplary, but not limiting serine proteases, include the following known serine protease inhibitors are used in the screening assays: Serine Protease Inhibitor 3 (SPI-3) (Chen, M. C., et al., *Citokine*, 11(11):856-862 (1999)); Aprotinin (Iijima, R., et al., *J. Biochem. (Tokyo)*, 126(5):912-916 (1999)); Kazal-type serine protease inhibitor-like proteins (Niimi, T., et al., *Eur. J. Biochem.*, 266(1):282-292 (1999)); Kunitz-type serine protease inhibitor (Ravichandran, S., et al., *Acta Crystallogr. D. Biol. Crystallogr.*, 55(11):1814-1821 (1999)); Tissue factor pathway inhibitor-2/Matrix-associated serine protease inhibitor (TFPI-2/MSPI), (Liu, Y., et al., *Arch. Biochem. Biophys.*, 370(1):112-8 (1999)); Bukunin, (Cui, C. Y., et al., *J. Invest.*

- Dermatol.*, 113(2):182-8 (1999)); Nafmostat mesilate (Ryo, R., et al., *Vox Sang.*, 76(4):241-6 (1999)); TPCK (Huang, Y., et al., *Oncogene*, 18(23):3431-9 (1999)); A synthetic cotton-bound serine protease inhibitor (Edwards, J. V., et al., *Wound Repair Regen.*, 7(2):106-18 (1999)); FUT-175 (Sawada, M., et al., *Stroke*, 30(3):644-50 (1999)); Combination of serine protease inhibitor FUT-0175 and thromboxane synthetase inhibitor OKY-046 (Kaminogo, M., et al., *Neurol. Med. Chir. (Tokyo)*, 38(11):704-8; discussion 708-9 (1998)); The rat serine protease inhibitor 2.1 gene (LeCam, A., et al., *Biochem. Biophys. Res. Commun.*, 253(2):311-4 (1998)); A new intracellular serine protease inhibitor expressed in the rat pituitary gland complexes with granzyme B (Hill, R. M., et al., *FEBS Lett.*, 440(3):361-4 (1998)); 3,4-Dichloroisocoumarin (Hammed, A., et al., *Proc. Soc. Exp. Biol. Med.*, 219(2):132-7 (1998)); LEX032 (Bains, A. S., et al., *Eur. J. Pharmacol.*, 356(1):67-72 (1998)); N-tosyl-L-phenylalanine chloromethyl ketone (Dryjanski, M., et al., *Biochemistry*, 37(40):14151-6 (1998)); Mouse gene for the serine protease inhibitor neuroserpin (P112) (Berger, P., et al., *Gene*, 214(1-2):25-33 (1998)); Rat serine protease inhibitor 2.3 gene (Paul, C., et al., *Eur. J. Biochem.*, 254(3):538-46 (1998)); Ecotin (Yang, S. Q., et al., *J. Mol. Biol.*, 279(4):945-57 (1998)); A 14 kDa plant-related serine protease inhibitor (Roch, P., et al., *Dev. Comp. Immunol.*, 22(1):1-12 (1998)); Matrix-associated serine protease inhibitor TFPI-2/33 kDa MSPI (Rao, C. N., et al., *Int. J. Cancer*, 76(5):749-56 (1998)); ONO-3403 (Hiwasa, T., et al., *Cancer Lett.*, 126(2):221-5 (1998)); Bdelastasin (Moser, M., et al., *Eur. J. Biochem.*, 253(1):212-20 (1998)); Bikunin (Xu, Y., et al., *J. Mol. Biol.*, 276(5):955-66 (1998)); Nafamostat mesilate (Meligren, K., et al., *Thromb. Haemost.*, 79(2):342-7 (1998)); The growth hormone dependent serine protease inhibitor, Spi 2.1 (Maake, C., et al., *Endocrinology*, 138(12):5630-6 (1997)); Growth factor activator inhibitor type 2, a Kunitz-type serine protease inhibitor (Kawaguchi, T., et al., *J. Biol. Chem.*, 272(44):27558-64 (1997)); Heat-stable serine protease inhibitor protein from ovaries of the desert locust, *Schistocerca gregaria* (Hamdaoui, A., et al., *Biochem. Biophys. Res. Commun.*, 238(2):357-60 (1997)); Bikunin, (Delaria, K. A., et al., *J. Biol. Chem.*, 272(18):12209-14 (1997)); Human placental bikunin (Marlor, C. W., et al., *J. Biol. Chem.*, 272(10):12202-8 (1997)); Hepatocyte growth factor activator inhibitor, a novel Kunitz-type serine protease inhibitor (Shimomura, T., et al., *J. Biol. Chem.*, 272(10):6370-6 (1997)); FUT-187, oral serine protease inhibitor, (Shiozaki, H., et al., *Gan To Kagaku Ryoho*, 23(14): 1971-9 (1996)); Extracellular matrix-associated serine protease inhibitors (Mr 33,000, 31,000, and 27,000 (Rao, C. N., et al., *Arch. Biochem. Biophys.*, 335(1):82-92 (1996)); An irreversible isocoumarin serine protease inhibitor (Palencia, D. D., et al., *Biol. Reprod.*, 55(3):536-42 (1996)); 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) (Nakabo, Y., et al., *J. Leukoc. Biol.*, 60(3):328-36 (1996)); Neuroserpin (Osterwalder, T., et al., *EMBO J.*, 15(12):2944-53 (1996)); Human serine protease inhibitor alpha-1-antitrypsin (Forney, J. R., et al., *J. Parasitol.* 82(3):496-502 (1996)); Rat serine protease inhibitor 2.3 (Simar-Blanchet, A. E., et al., *Eur. J. Biochem.*, 236(2):638-48 (1996)); Gebaxate mesilate (parodi, F., et al., *J. Cardiothorac. Vasc. Anesth.*, 10(2):235-7 (1996)); Recombinant serine protease inhibitor, CPTI II (Stankiewicz, M., et al., *Acta Biochim. Pol.*, 43(3):525-9 (1996)); A cysteine-rich serine protease inhibitor (Guamerin II) (Kim, D. R., et al., *J. Enzym. Inhib.*, 10(2):81-91 (1996)); Diisopropylfluorophosphate (Lundqvist, H., et al., *Inflamm. Res.*, 44(12):510-7 (1995)); Nexin 1 (Yu, D. W., et al., *J. Cell Sci.*, 108(Pt 12):3867-74 (1995)); LEX032 (Scalia, R., et al., *Shock*, 4(4):251-6 (1995)); Protease nexin I (Houenou, L. J., et al., *Proc. Natl. Acad. Sci. U.S.A.*, 92(3):895-9 (1995)); Chymase-directed serine protease inhibitor (Woodard S. L., et al., *J. Immunol.*, 153(11):5016-25 (1994)); N-alpha-tosyl-L-lysyl-chloromethyl ketone (TLCK) (Bourinbaier, A. S., et al., *Cell Immunol.*, 155(1):230-6 (1994)); Smpi56 (Ghendler, Y., et al., *Exp. Parasitol.*, 78(2):121-31 (1994)); Schistosoma haematobium serine protease (Blanton, R. E., et al., *Mol. Biochem. Parasitol.*, 63(1):1-11 (1994)); Spi-1 (Warren, W. C., et al., *Mol. Cell Endocrinol.*, 98(1):27-32 (1993)); TAME (Jessop, J. J., et al., *Inflammation*, 17(5):613-31 (1993)); Antithrombin III (Kalaria, R. N., et al., *Am. J. Pathol.*, 143(3):886-93 (1993)); FOY-305 (Ohkoshi, M., et al., *Anticancer Res.*, 13(4):963-6 (1993)); Camostat mesilate (Senda, S., et al., *Intern. Med.*, 32(4):350-4 (1993)); Pigment epithelium-derived factor (Steele, F. R., et al., *Proc. Natl. Acad. Sci. U.S.A.*, 90(4):1526-30 (1993)); Antistatin (Holstein, T. W., et al., *FEBS Lett.*, 309(3):288-92 (1992)); The vaccinia virus K2L gene encodes a serine protease inhibitor (Zhou, J., et al., *Virology*, 189(2):678-86 (1992)); Bowman-Birk serine-protease inhibitor (Werner, M. H., et al., *J. Mol. Biol.*, 225(3):873-89 (1992)); FUT-175 (Yanamoto, H., et al., *Neurosurgery*, 30(3):358-63 (1992)); FUT-175; (Yanamoto, H., et al., *Neurosurgery*, 30(3):351-6, discussion 356-7 (1992)); PAI-I (Yreadwell, B. V., et al., *J. Orthop. Res.*, 9(3):309-16 (1991)); 3,4-Dichloroisocoumarin (Rusbridge, N. M., et al., *FEBS Lett.*, 268(1):133-6 (1990)); Alpha 1-antichymotrypsin (Lindmark, B. E., et al., *Am. Rev. Respir. Des.*, 141(4 Pt 1):884-8 (1990)); P-toluenesulfonyl-L-arginine methyl ester (TAME) (Scuderi, P., *J. Immunol.*, 143(1):168-73 (1989)); Aprotinin (Seto, S., et al., *Adv. Exp. Med. Biol.*, 247B:49-54 (1989)); Alpha 1-antichymotrypsin (Abraham, C. R., et al., *Cell*, 52(4):487-501 (1988)); Contrapsin (Modha, J., et al., *Parasitology*, 96 (Pt 1):99-109 (1988)); (FOY-305) (Yamauchi, Y., et al., *Hiroshima J. Med. Sci.*, 36(1):81-7 No abstract available (1987)); Alpha 2-antiplasmin (Holmes, W. E., et al., *J. Biol. Chem.*, 262(4):1659-64 (1987)); 3,4-dichloroisocoumarin (Harper, J. W., et al., *Biochemistry*, 24(8):1831-41 (1985)); Diisopropylfluorophosphate (Tsutsui, K., et al., *Biochem. Biophys. Res. Commun.*, 123(1):271-7 (1984)); Gabexate mesilate (Hesse, B., et al., *Pharmacol Res. Commun.*, 16(7):637-45 (1984)); Phenyl methyl sulfonyl fluoride (Dufer, J., et al., *Scand. J. Haematol.*, 32(1):25-32 (1984)); Aprotinin (Seto, S., et al., *Hypertension*, 5(6):893-9 (1983)); Protease inhibitor CI-2 (McPhalen, C. A., et al., *J. Mol. Biol.*, 168(2):445-7 (1983)); Phenylmethylsulfonyl fluoride (Sekar V., et al., *Biochem. Biophys. Res. Commun.*, 89(2):474-8 (1979)); PGE1 (Feinstein, M. D., et al., *Prostaglandine*, 14(6):1075-93 (1977)
- [0387]** C. Combinatorial Libraries and Other Libraries
- [0388]** The source of compounds for the screening assays, can be libraries, including, but are not limited to, combinatorial libraries. Methods for synthesizing combinatorial libraries and characteristics of such combinatorial libraries are known in the art (See generally, *Combinatorial Libraries: Synthesis, Screening and Application Potential* (Cortese Ed.) Walter de Gruyter, Inc., 1995; Tietze and Lieb, *Curr. Opin. Chem. Biol.*, 2(3):363-71 (1998); Lam, *Anticancer Drug Des.*, 12(3):145-67 (1997); Blaney and Martin, *Curr.*

*Opin. Chem. Biol.*, 1(1):54-9 (1997); and Schultz and Schultz, *Biotechnol. Prog.*, 12(6):729-43 (1996)).

[0389] Methods and strategies for generating diverse libraries, primarily peptide- and nucleotide-based oligomer libraries, have been developed using molecular biology methods and/or simultaneous chemical synthesis methodologies (see, e.g., Dower et al., *Annu. Rep. Med. Chem.*, 26:271-280 (1991); Fodor et al., *Science*, 251:767-773 (1991); Jung et al., *Angew. Chem. Int. Ed. Engl.*, 31:367-383 (1992); Zuckerman et al., *Proc. Natl. Acad. Sci. USA*, 89:4505-4509 (1992); Scott et al., *Science*, 249:386-390 (1990); Devlin et al., *Science*, 249:404-406 (1990); Cwirla et al., *Proc. Natl. Acad. Sci. USA*, 87:6378-6382 (1990); and Gallop et al., *J. Medicinal Chemistry*, 37:1233-1251 (1994)). The resulting combinatorial libraries potentially contain millions of compounds and that can be screened to identify compounds that exhibit a selected activity.

[0390] The libraries fall into roughly three categories: fusion-protein-displayed peptide libraries in which random peptides or proteins are presented on the surface of phage particles or proteins expressed from plasmids; support-bound synthetic chemical libraries in which individual compounds or mixtures of compounds are presented on insoluble matrices, such as resin beads (see, e.g., Lam et al., *Nature*, 354:82-84 (1991)) and cotton supports (see, e.g., Eichler et al., *Biochemistry* 32:11035-11041 (1993)); and methods in which the compounds are used in solution (see, e.g., Houghten et al., *Nature*, 354:84-86 (1991); Houghten et al., *BioTechniques*, 313:412-421 (1992); and Scott et al., *Curr. Opin. Biotechnol.*, 5:40-48 (1994)). There are numerous examples of synthetic peptide and oligonucleotide combinatorial libraries and there are many methods for producing libraries that contain non-peptidic small organic molecules. Such libraries can be based on basis set of monomers that are combined to form mixtures of diverse organic molecules or that can be combined to form a library based upon a selected pharmacophore monomer.

[0391] Either a random or a deterministic combinatorial library can be screened by the presently disclosed and/or claimed screening methods. In either of these two libraries, each unit of the library is isolated and/or immobilized on a solid support. In the deterministic library, one knows a priori a particular unit's location on each solid support. In a random library, the location of a particular unit is not known a priori although each site still contains a single unique unit. Many methods for preparing libraries are known to those of skill in this art (see, e.g., Geysen et al., *Proc. Natl. Acad. Sci. USA*, 81:3998-4002 (1984), Houghten et al., *Proc. Natl. Acad. Sci. USA*, 81:5131-5135 (1985)). Combinatorial library generated by the any techniques known to those of skill in the art are contemplated (see, e.g., Table 1 of Schultz and Schultz, *Biotechnol. Prog.*, 12(6):729-43 (1996)) for screening; Bartel et al., *Science*, 261:1411-1418 (1993); Baumbach et al. *BioPharm*, (May):24-35 (1992); Bock et al. *Nature*, 355:564-566 (1992); Borman, S., Combinatorial chemists focus on small molecules molecular recognition, and automation, *Chem. Eng. News*, 2(12):29 (1996); Boublik, et al., Eukaryotic Virus Display: Engineering the Major Surface Glycoproteins of the Autographa California Nuclear Polyhedrosis Virus (ACNPV) for the Presentation of Foreign Proteins on the Virus Surface, *Bio/Technology*, 13:1079-1084 (1995); Brenner, et al., Encoded Combinatorial Chemistry, *Proc. Natl. Acad. Sci. U.S.A.*, 89:5381-5383

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- [0392] For example, peptides that bind to an MTSP protein or a protease domain of an MTSP protein can be identified using phage display libraries. In an exemplary embodiment, this method can include a) contacting phage from a phage library with the MTSP protein or a protease domain thereof; (b) isolating phage that bind to the protein; and (c) determining the identity of at least one peptide coded by the isolated phage to identify a peptide that binds to an MTSP protein.
- [0393] H. Modulators of the Activity of MTSP Proteins
- [0394] Provided herein are compounds, identified by screening or produced using the MTSP proteins or protease domain in other screening methods, that modulate the activity of an MTSP. These compounds act by directly interacting with the MTSP protein or by altering transcription or translation thereof. Such molecules include, but are not limited to, antibodies that specifically react with an MTSP protein, particularly with the protease domain thereof, antisense nucleic acids that alter expression of the MTSP protein, antibodies, peptide mimetics and other such compounds.
- [0395] 1. Antibodies
- [0396] Antibodies, including polyclonal and monoclonal antibodies, that specifically bind to the MTSP proteins provided herein, particularly to the single chain protease domains thereof are provided. Preferably, the antibody is a monoclonal antibody, and preferably, the antibody specifically binds to the protease domain of the MTSP protein. In particular embodiments, antibodies to each of the single chain of protease domain of MTSP1, MTSP3, MTSP4 and MTSP6 Also provided are antibodies that specifically bind to any domain of MTSP3 or MTSP4, and to double chain forms thereof.
- [0397] The MTSP protein and domains, fragments, homologs and derivatives thereof may be used as immunogens to generate antibodies that specifically bind such immunogens. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a specific embodiment, antibodies to human MTSP protein are produced. In another embodiment, complexes formed from fragments of MTSP protein, which fragments contain the serine protease domain, are used as immunogens for antibody production.
- [0398] Various procedures known in the art may be used for the production of polyclonal antibodies to MTSP protein,

its domains, derivatives, fragments or analogs. For production of the antibody, various host animals can be immunized by injection with the native MTSP protein or a synthetic version, or a derivative of the foregoing, such as a cross-linked MTSP protein. Such host animals include but are not limited to rabbits, mice, rats, etc. Various adjuvants can be used to increase the immunological response, depending on the host species, and include but are not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, and potentially useful human adjuvants such as bacille Calmette-Guerin (BCG) and corynebacterium parvum.

[0399] For preparation of monoclonal antibodies directed towards an MTSP protein or domains, derivatives, fragments or analogs thereof, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. Such techniques include but are not restricted to the hybridoma technique originally developed by Kohler and Milstein (*Nature* 256:495-497 (1975)), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., *Immunology Today* 4:72 (1983)), and the EBV hybridoma technique to produce human monoclonal antibodies (Cole et al., in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)). In an additional embodiment, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). Human antibodies may be used and can be obtained by using human hybridomas (Cote et al., *Proc. Natl. Acad. Sci. USA* 80:2026-2030 (1983)). Or by transforming human B cells with EBV virus in vitro (Cole et al., in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)). Techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Neuberger et al., *Nature* 312:604-608 (1984); Takeda et al., *Nature* 314:452-454 (1985)) by splicing the genes from a mouse antibody molecule specific for the MTSP protein together with genes from a human antibody molecule of appropriate biological activity can be used.

[0400] Techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce MTSP protein-specific single chain antibodies. An additional embodiment uses the techniques described for the construction of Fab expression libraries (Huse et al., *Science* 246:1275-1281 (1989)) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for MTSP protein or MTSP protein, or domains, derivatives, or analogs thereof. Non-human antibodies can be "humanized" by known methods (see, e.g., U.S. Pat. No. 5,225,539).

[0401] Antibody fragments that contain the idiotypes of MTSP protein can be generated by techniques known in the art. For example, such fragments include but are not limited to: the F(ab')<sub>2</sub> fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments that can be generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragment, the Fab fragments that can be generated by treating the antibody molecular with papain and a reducing agent, and Fv fragments.

[0402] In the production of antibodies, screening for the desired antibody can be accomplished by techniques known

in the art, e.g., ELISA (enzyme-linked immunosorbent assay). To select antibodies specific to a particular domain of the MTSP protein one may assay generated hybridomas for a product that binds to the fragment of the MTSP protein that contains such a domain

[0403] The foregoing antibodies can be used in methods known in the art relating to the localization and/or quantitation of MTSP protein proteins, e.g., for imaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

[0404] In another embodiment, (see *infra*), anti-MTSP protein antibodies, or fragments thereof, containing the binding domain are used as therapeutic agents.

[0405] 2. Peptides and Peptide Mimetics

[0406] Provided herein are methods for identifying molecules that bind to and modulate the activity of MTSP proteins. Included among molecules that bind to MTSPs, particularly the single chain protease domain or catalytically active fragments thereof, are peptides and peptide mimetics. Peptide mimetics are molecules or compounds that mimic the necessary molecular conformation of a ligand or polypeptide for specific binding to a target molecule such as, e.g., an MTSP protein. In an exemplary embodiment, the peptides or peptide mimetics bind to the protease domain of the MTSP protein. Such peptides and peptide mimetics include those of antibodies that specifically bind an MTSP protein and, preferably, bind to the protease domain of an MTSP protein. The peptides and peptide mimetics identified by methods provided herein can be agonists or antagonists of MTSP proteins.

[0407] Such peptides and peptide mimetics are useful for diagnosing, treating, preventing, and screening for a disease or disorder associated with MTSP protein activity in a mammal. In addition, the peptides and peptide mimetics are useful for identifying, isolating, and purifying molecules or compounds that modulate the activity of an MTSP protein, or specifically bind to an MTSP protein, preferably, the protease domain of an MTSP protein. Low molecular weight peptides and peptide mimetics can have strong binding properties to a target molecule, e.g., an MTSP protein or, preferably, to the protease domain of an MTSP protein.

[0408] Peptides and peptide mimetics that bind to MTSP proteins as described herein can be administered to mammals, including humans, to modulate MTSP protein activity. Thus, methods for therapeutic treatment and prevention of neoplastic diseases comprise administering a peptide or peptide mimetic compound in an amount sufficient to modulate such activity are provided. Thus, also provided herein are methods for treating a subject having such a disease or disorder in which a peptide or peptide mimetic compound is administered to the subject in a therapeutically effective dose or amount.

[0409] Compositions containing the peptides or peptide mimetics can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions can be administered to a patient already suffering from a disease, as described above, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. Amounts effective for this use will depend on the severity of the disease and the weight and general state of the patient.

[0410] In prophylactic applications, compositions containing the peptides and peptide mimetics are administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be a "prophylactically effective dose". In this use, the precise amounts again depend on the patient's state of health and weight.

[0411] Accordingly, the peptides and peptide mimetics that bind to an MTSP protein can be used generating pharmaceutical compositions containing, as an active ingredient, at least one of the peptides or peptide mimetics in association with a pharmaceutical carrier or diluent. The compounds can be administered, for example, by oral, pulmonary, parental (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), inhalation (via a fine powder formulation), transdermal, nasal, vaginal, rectal, or sublingual routes of administration and can be formulated in dosage forms appropriate for each route of administration (see, e.g., International PCT application Nos. WO 93/25221 and WO 94/17784; and European Patent Application 613, 683).

[0412] Peptides and peptide mimetics that bind to MTSP proteins are useful in vitro as unique tools for understanding the biological role of MTSP proteins, including the evaluation of the many factors thought to influence, and be influenced by, the production of MTSP protein. Such peptides and peptide mimetics are also useful in the development of other compounds that bind to and modulate the activity of an MTSP protein, because such compounds provide important information on the relationship between structure and activity that should facilitate such development.

[0413] The peptides and peptide mimetics are also useful as competitive binders in assays to screen for new MTSP proteins or MTSP protein agonists. In such assay embodiments, the compounds can be used without modification or can be modified in a variety of ways; for example, by labeling, such as covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the materials thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups such as: radiolabels such as <sup>125</sup>I enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups. The compounds may also include spacers or linkers in cases where the compounds are to be attached to a solid support.

[0414] Moreover, based on their ability to bind to an MTSP protein, the peptides and peptide mimetics can be used as reagents for detecting MTSP proteins in living cells, fixed cells, in biological fluids, in tissue homogenates, in purified, natural biological materials, etc. For example, by labelling such peptides and peptide mimetics, one can identify cells having MTSP proteins. In addition, based on their ability to bind an MTSP protein, the peptides and peptide mimetics can be used in situ staining, FACS (fluorescence-activated cell sorting), Western blotting, ELISA, etc. In addition, based on their ability to bind to an MTSP protein, the peptides and peptide mimetics can be used in purification

of MTSP protein polypeptides or in purifying cells expressing the MTSP protein polypeptides, e.g., a polypeptide encoding the protease domain of an MTSP protein.

[0415] The peptides and peptide mimetics can also be used as commercial reagents for various medical research and diagnostic uses.

[0416] The activity of the peptides and peptide mimetics can be evaluated either in vitro or in vivo in one of the numerous models described in McDonald (1992) *Am. J. of Pediatric Hematology/Oncology*, 14:8-21, which is incorporated herein by reference.

### [0417] 3. Peptide and Peptide Mimetic Therapy

[0418] Peptides and peptide mimetics that can bind to MTSP proteins or the protease domain of MTSP proteins and modulate the activity thereof, or have MTSP protein activity, can be used for treatment of neoplastic. The peptides and peptide mimetics may be delivered, in vivo or ex vivo, to the cells of a subject in need of treatment. Further, peptides which have MTSP protein activity can be delivered, in vivo or ex vivo, to cells which carry mutant or missing alleles encoding the MTSP protein gene. Any of the techniques described herein or known to the skilled artisan can be used for preparation and in vivo or ex vivo delivery of such peptides and peptide mimetics that are substantially free of other human proteins. For example, the peptides can be readily prepared by expression in a microorganism or synthesis in vitro.

[0419] The peptides or peptide mimetics can be introduced into cells, in vivo or ex vivo, by microinjection or by use of liposomes, for example. Alternatively, the peptides or peptide mimetics may be taken up by cells, in vivo or ex vivo, actively or by diffusion. In addition, extracellular application of the peptide or peptide mimetic may be sufficient to effect treatment of a neoplastic disease. Other molecules, such as drugs or organic compounds, that: 1) bind to an MTSP protein or protease domain thereof; or 2) have a similar function or activity to an MTSP protein or protease domain thereof, may be used in methods for treatment.

### [0420] 4. Rational Drug Design

[0421] The goal of rational drug design is to produce structural analogs of biologically active polypeptides or peptides of interest or of small molecules or peptide mimetics with which they interact (e.g., agonists, antagonists, inhibitors) in order to fashion drugs which are, e.g., more active or stable forms thereof; or which, e.g., enhance or interfere with the function of a polypeptide in vivo (e.g., an MTSP protein). In one approach, one first determines the three-dimensional structure of a protein of interest (e.g., an MTSP protein or polypeptide having a protease domain) or, for example, of a MTSP protein-ligand complex, by X-ray crystallography, by computer modeling or most typically, by a combination of approaches (see, e.g., Erickson et al. 1990). Also, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. In addition, peptides can be analyzed by an alanine scan. In this technique, an amino acid residue is replaced by Ala, and its effect on the peptide's activity is determined. Each of the amino acid residues of the peptide is analyzed in this manner to determine the important regions of the peptide.

[0422] Also, a polypeptide or peptide that binds to an MTSP protein or, preferably, the protease domain of an MTSP protein, can be selected by a functional assay, and then the crystal structure of this polypeptide or peptide can be determined. The polypeptide can be, for example, an antibody specific for an MTSP protein or the protein domain of an MTSP protein. This approach can yield a pharmacore upon which subsequent drug design can be based. Further, it is possible to bypass the crystallography altogether by generating anti-idiotypic polypeptides or peptides, (anti-ids) to a functional, pharmacologically active polypeptide or peptide that binds to an MTSP protein or protease domain of an MTSP protein. As a mirror image of a mirror image, the binding site of the anti-ids is expected to be an analog of the original target molecule, e.g., an MTSP protein or polypeptide having an MTSP protein. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced banks of peptides. Selected peptides would then act as the pharmacore.

[0423] Thus, one may design drugs which have, e.g., improved activity or stability or which act as modulators (e.g., inhibitors, agonists, antagonists, etc.) of MTSP protein activity, and are useful in the methods, particularly the methods for diagnosis, treatment, prevention, and screening of a neoplastic disease. By virtue of the availability of cloned MTSP protein sequences, sufficient amounts of the MTSP protein polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, the knowledge of the amino acid sequence of an MTSP protein or the protease domain thereof, e.g., the protease domain encoded by the amino acid sequence of SEQ ID NO: 2, can provide guidance on computer modeling techniques in place of, or in addition to, X-ray crystallography.

[0424] Methods of Identifying Peptides and Peptide Mimetics that Bind to MTSP Proteins

[0425] Peptides having a binding affinity to the MTSP protein polypeptides provided herein (e.g., an MTSP protein or a polypeptide having a protease domain of an MTSP protein) can be readily identified, for example, by random peptide diversity generating systems coupled with an affinity enrichment process. Specifically, random peptide diversity generating systems include the "peptides on plasmids" system (see, e.g., U.S. Pat. Nos. 5,270,170 and 5,338,665); the "peptides on phage" system (see, e.g., U.S. Pat. No. 6,121,238 and Cwirla, et al. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87:6378-6382); the "polysome system;" the "encoded synthetic library (ESL)" system; and the "very large scale immobilized polymer synthesis" system (see, e.g., U.S. Pat. No. 6,121,238; and Dower et al. (1991) *An. Rep. Med. Chem.* 26:271-280

[0426] For example, using the procedures described above, random peptides can generally be designed to have a defined number of amino acid residues in length (e.g., 12). To generate the collection of oligonucleotides encoding the random peptides, the codon motif (NNK)<sub>x</sub>, where N is nucleotide A, C, G, or T (equimolar; depending on the methodology employed, other nucleotides can be employed), K is G or T (equimolar), and x is an integer corresponding to the number of amino acids in the peptide (e.g., 12) can be used to specify any one of the 32 possible codons resulting from the NNK motif: 1 for each of 12

amino acids, 2 for each of 5 amino acids, 3 for each of 3 amino acids, and only one of the three stop codons. Thus, the NNK motif encodes all of the amino acids, encodes only one stop codon, and reduces codon bias.

[0427] The random peptides can be presented, for example, either on the surface of a phage particle, as part of a fusion protein containing either the pIII or the pVIII coat protein of a phage fd derivative (peptides on phage) or as a fusion protein with the LacI peptide fusion protein bound to a plasmid (peptides on plasmids). The phage or plasmids, including the DNA encoding the peptides, can be identified and isolated by an affinity enrichment process using immobilized MTSP protein polypeptide having a protease domain. The affinity enrichment process, sometimes called "panning," typically involves multiple rounds of incubating the phage, plasmids, or polysomes with the immobilized MTSP protein polypeptide, collecting the phage, plasmids, or polysomes that bind to the MTSP protein polypeptide (along with the accompanying DNA or mRNA), and producing more of the phage or plasmids (along with the accompanying LacI-peptide fusion protein) collected.

[0428] Characteristics of Peptides and Peptide Mimetics

[0429] Typically, the molecular weight of preferred peptides or peptide mimetics is from about 250 to about 8,000 daltons. If the peptides are oligomerized, dimerized and/or derivatized with a hydrophilic polymer (e.g., to increase the affinity and/or activity of the compounds), the molecular weights of such peptides can be substantially greater and can range anywhere from about 500 to about 120,000 daltons, more preferably from about 8,000 to about 80,000 daltons. Such peptides can comprise 9 or more amino acids wherein the amino acids are naturally occurring or synthetic (non-naturally occurring) amino acids. One skilled in the art would know how to determine the affinity and molecular weight of the peptides and peptide mimetics suitable for therapeutic and/or diagnostic purposes (e.g., see Dower et al., U.S. Pat. No. 6,121,238).

[0430] The peptides may be covalently attached to one or more of a variety of hydrophilic polymers. Suitable hydrophilic polymers include, but are not limited to, polyalkylenes as exemplified by polyethylene glycol and polypropylene glycol, polylactic acid, polyglycolic acid, polyoxyalkenes, polyvinylalcohol, polyvinylpyrrolidone, cellulose and cellulose derivatives, dextran and dextran derivatives, etc. When the peptide compounds are derivatized with such polymers, their solubility and circulation half-lives can be increased with little, if any, diminishment in their binding activity. The peptide compounds may be dimerized and each of the dimeric subunits can be covalently attached to a hydrophilic polymer. The peptide compounds can be PEGylated, i.e., covalently attached to polyethylene glycol (PEG).

[0431] Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics" (Luthman et al., *A Textbook of Drug Design and Development*, 14:386-406, 2nd Ed., Harwood Academic Publishers (1996); Joachim Grante (1994) *Angew. Chem. Int. Ed. Engl.*, 33:1699-1720; Fauchere (1986) *J. Adv. Drug Res.*, 15:29; Veber and Freidinger (1985) *TINS*, p. 392; and Evans et al. (1987) *J. Med. Chem.* 30:1229). Peptide

mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Preparation of peptidomimetics and structures thereof are known to those of skill in this art.

[0432] Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides containing a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo et al. (1992) *An. Rev. Biochem.*, 61:387, incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

[0433] Those skilled in the art would appreciate that modifications may be made to the peptides and mimetics without deleteriously effecting the biological or functional activity of the peptide. Further, the skilled artisan would know how to design non-peptide structures in three dimensional terms, that mimic the peptides that bind to a target molecule, e.g., an MTSP protein or, preferably, the protease domain of MTSP proteins (see, e.g., Eck and Sprang (1989) *J. Biol. Chem.*, 26: 17605-18795).

[0434] When used for diagnostic purposes, the peptides and peptide mimetics may be labeled with a detectable label and, accordingly, the peptides and peptide mimetics without such a label can serve as intermediates in the preparation of labeled peptides and peptide mimetics. Detectable labels can be molecules or compounds, which when covalently attached to the peptides and peptide mimetics, permit detection of the peptide and peptide mimetics in vivo, for example, in a patient to whom the peptide or peptide mimetic has been administered, or in vitro, e.g., in a sample or cells. Suitable detectable labels are well known in the art and include, by way of example, radioisotopes, fluorescent labels (e.g., fluorescein), and the like. The particular detectable label employed is not critical and is selected relative to the amount of label to be employed as well as the toxicity of the label at the amount of label employed. Selection of the label relative to such factors is well within the skill of the art.

[0435] Covalent attachment of a detectable label to the peptide or peptide mimetic is accomplished by conventional methods well known in the art. For example, when the <sup>125</sup>I radioisotope is employed as the detectable label, covalent attachment of <sup>125</sup>I to the peptide or the peptide mimetic can be achieved by incorporating the amino acid tyrosine into the peptide or peptide mimetic and then iodinating the peptide (see, e.g., Weaner et al. (1994) *Synthesis and Applications of Isotopically Labelled Compounds*, pp. 137-140). If tyrosine is not present in the peptide or peptide mimetic, incorporation of tyrosine to the N or C terminus of the peptide or peptide mimetic can be achieved by well known chemistry. Likewise, <sup>32</sup>P can be incorporated onto the peptide or peptide mimetic as a phosphate moiety through, for example, a hydroxyl group on the peptide or peptide mimetic using conventional chemistry.

[0436] Labeling of peptidomimetics usually involves covalent attachment of one or more labels, directly or through a spacer (e.g., an amide group), to non-interfering position(s) on the peptidomimetic that are predicted by

quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with the macromolecule(s) to which the peptidomimetic binds to produce the therapeutic effect. Derivatization (e.g., labeling) of peptidomimetics should not substantially interfere with the desired biological or pharmacological activity of the peptidomimetic.

#### [0437] 6. Methods of Preparing Peptides and Peptide Mimetics

[0438] Peptides that bind to MTSP proteins can be prepared by classical methods known in the art, for example, by using standard solid phase techniques. The standard methods include exclusive solid phase synthesis, partial solid phase synthesis methods, fragment condensation, classical solution synthesis, and even by recombinant DNA technology (see, e.g., Merrifield (1963) *J. Am. Chem. Soc.*, 85:2149, incorporated herein by reference.)

[0439] Using the "encoded synthetic library" or "very large scale immobilized polymer synthesis" systems (see, e.g., U.S. Pat. Nos. 5,925,525, and 5,902,723); one can not only determine the minimum size of a peptide with the activity of interest, one can also make all of the peptides that form the group of peptides that differ from the preferred motif (or the minimum size of that motif) in one, two, or more residues. This collection of peptides can then be screened for ability to bind to the target molecule, e.g., and MTSP protein or, preferably, the protease domain of an MTSP protein. This immobilized polymer synthesis system or other peptide synthesis methods can also be used to synthesize truncation analogs and deletion analogs and combinations of truncation and deletion analogs of the peptide compounds.

[0440] These procedures can also be used to synthesize peptides in which amino acids other than the 20 naturally occurring, genetically encoded amino acids are substituted at one, two, or more positions of the peptide. For instance, naphthylalanine can be substituted for tryptophan, facilitating synthesis. Other synthetic amino acids that can be substituted into the peptides include L-hydroxypropyl, L-3, 4-dihydroxy-phenylalanyl, D amino acids such as L-d-hydroxylysyl and D-d-methylalanyl, L- $\alpha$ -methylalanyl,  $\beta$  amino acids, and isoquinolyl. D amino acids and non-naturally occurring synthetic amino acids can also be incorporated into the peptides (see, e.g., Roberts et al. (1983) *Unusual AminoAcids in Peptide Synthesis*, 5(6):341-449).

[0441] The peptides may also be modified by phosphorylation (see, e.g., W. Bannwarth et al. (1996) *Biorganic and Medicinal Chemistry Letters*, 6(17):2141-2146), and other methods for making peptide derivatives (see, e.g., Hruby et al. (1990) *Biochem. J.*, 268(2):249-262). Thus, peptide compounds also serve as a basis to prepare peptide mimetics with similar biological activity.

[0442] Those of skill in the art recognize that a variety of techniques are available for constructing peptide mimetics with the same or similar desired biological activity as the corresponding peptide compound but with more favorable activity than the peptide with respect to solubility, stability, and susceptibility to hydrolysis and proteolysis (see, e.g., Morgan et al. (1989) *An. Rep. Med. Chem.*, 24:243-252). Methods for preparing peptide mimetics modified at the N-terminal amino group, the C-terminal carboxyl group,

and/or changing one or more of the amido linkages in the peptide to a non-amido linkage are known to those of skill in the art.

[0443] Amino terminus modifications include alkylating, acetylating, adding a carbobenzoyle group, forming a succinimide group, etc. (see, e.g., Murray et al. (1995) *Burger's Medicinal Chemistry and Drug Discovery*, 5th ed., Vol. 1, Manfred E. Wolf, ed., John Wiley and Sons, Inc.). C-terminal modifications include mimetics wherein the C-terminal carboxyl group is replaced by an ester, an amide or modifications to form a cyclic peptide.

[0444] In addition to N-terminal and C-terminal modifications, the peptide compounds, including peptide mimetics, can advantageously be modified with or covalently coupled to one or more of a variety of hydrophilic polymers. It has been found that when peptide compounds are derivatized with a hydrophilic polymer, their solubility and circulation half-lives may be increased and their immunogenicity is masked, with little, if any, diminishment in their binding activity. Suitable nonproteinaceous polymers include, but are not limited to, polyalkylethers as exemplified by polyethylene glycol, polypropylene glycol, polylactic acid, polyglycolic acid, polyoxyalkenes, polyvinylalcohol, polyvinylpyrrolidone, cellulose and cellulose derivatives, dextran and dextran derivatives, etc. Generally, such hydrophilic polymers have an average molecular weight ranging from about 500 to about 100,000 daltons, more preferably from about 2,000 to about 40,000 daltons and, even more preferably, from about 5,000 to about 20,000 daltons. The hydrophilic polymers also can have an average molecular weights of about 5,000 daltons, 10,000 daltons and 20,000 daltons.

[0445] Methods for derivatizing peptide compounds or for coupling peptides to such polymers have been described (see, e.g., Zallipsky (1995) *Bioconjugate Chem.*, 6:150-165; Monfardini et al. (1995) *Bioconjugate Chem.*, 6:62-69; U.S. Pat. No. 4,640,835; U.S. Pat. No. 4,496,689; U.S. Pat. No. 4,301,144; U.S. Pat. No. 4,670,417; U.S. Pat. No. 4,791,192; U.S. Pat. No. 4,179,337 and WO 95/34326, all of which are incorporated by reference in their entirety herein).

[0446] Other methods for making peptide derivatives are described, for example, in Hruby et al. (1990), *Biochem J.*, 268(2):249-262, which is incorporated herein by reference. Thus, the peptide compounds also serve as structural models for non-peptidic compounds with similar biological activity. Those of skill in the art recognize that a variety of techniques are available for constructing compounds with the same or similar desired biological activity as a particular peptide compound but with more favorable activity with respect to solubility, stability, and susceptibility to hydrolysis and proteolysis (see, e.g., Morgan et al. (1989) *An. Rep. Med. Chem.*, 24:243-252, incorporated herein by reference). These techniques include replacing the peptide backbone with a backbone composed of phosphonates, amidates, carbamates, sulfonamides, secondary amines, and N-methylamino acids.

[0447] Peptide compounds may exist in a cyclized form with an intramolecular disulfide bond between the thiol groups of the cysteines. Alternatively, an intermolecular disulfide bond between the thiol groups of the cysteines can be produced to yield a dimeric (or higher oligomeric) compound. One or more of the cysteine residues may also be substituted with a homocysteine.

#### [0448] I. Conjugates

[0449] A conjugate, containing: a) a single chain protease domain (or proteolytically active portion thereof) of an MTSP protein or an MTSP3, MTSP4 or MTSP6 full length zymogen, activated form thereof, or double or single chain protease domain thereof; and b) a targeting agent linked to the MTSP protein directly or via a linker, wherein the agent facilitates: i) affinity isolation or purification of the conjugate; ii) attachment of the conjugate to a surface; iii) detection of the conjugate; or iv) targeted delivery to a selected tissue or cell, is provided herein. The conjugate can be a chemical conjugate or a fusion protein mixture thereof.

[0450] The targeting agent is preferably a protein or peptide fragment, such as a tissue specific or tumor specific monoclonal antibody or growth factor or fragment thereof linked either directly or via a linker to an MTSP protein or a protease domain thereof. The targeting agent may also be a protein or peptide fragment that contains a protein binding sequence, a nucleic acid binding sequence, a lipid binding sequence, a polysaccharide binding sequence, or a metal binding sequence, or a linker for attachment to a solid support. In a particular embodiment, the conjugate contains a) the MTSP or portion thereof, as described herein; and b) a targeting agent linked to the MTSP protein directly or via a linker.

[0451] Conjugates, such as fusion proteins and chemical conjugates, of the MTSP protein with a protein or peptide fragment (or plurality thereof) that functions, for example, to facilitate affinity isolation or purification of the MTSP protein domain, attachment of the MTSP protein domain to a surface, or detection of the MTSP protein domain are provided. The conjugates can be produced by chemical conjugation, such as via thiol linkages, but are preferably produced by recombinant means as fusion proteins. In the fusion protein, the peptide or fragment thereof is linked to either the N-terminus or C-terminus of the MTSP protein domain. In chemical conjugates the peptide or fragment thereof may be linked anywhere that conjugation can be effected, and there may be a plurality of such peptides or fragments linked to a single MTSP protein domain or to a plurality thereof.

[0452] The targeting agent is preferably for in vitro delivery to a cell or tissue, and includes agents such as cell or tissue-specific antibodies, growth factors and other factors expressed on specific cells; and other cell or tissue specific agents that promote directed delivery of a linked protein.

[0453] Most preferably the targeting agent specifically delivers the MTSP protein to selected cells by interaction with a cell surface protein and internalization of conjugate or MTSP protein portion thereof. These conjugates are used in a variety of methods and are particularly suited for use in methods of activation of prodrugs, such as prodrugs that upon cleavage by the particular MTSP protein are cytotoxic. The prodrugs are administered prior to simultaneously with or subsequently to the conjugate. Upon delivery to the targeted cells, the protease activates the prodrug, which then exhibits its therapeutic effect, such as a cytotoxic effect.

#### [0454] 1. Conjugation

[0455] Conjugates with linked MTSP protein domains can be prepared either by chemical conjugation, recombinant DNA technology, or combinations of recombinant expres-

sion and chemical conjugation. The MTSP protein domains and the targeting agent may be linked in any orientation and more than one targeting agents and/or MTSP protein domains may be present in a conjugate.

[0456] a. Fusion Proteins

[0457] Fusion proteins are proved herein. A fusion protein contains: a) one or a plurality of domains of an MTSP proteins and b) a targeting agent. The fusion proteins are preferably produced by recombinant expression of nucleic acids that encode the fusion protein.

[0458] b. Chemical Conjugation

[0459] To effect chemical conjugation herein, the MTSP protein domain is linked via one or more selected linkers or directly to the targeting agent. Chemical conjugation must be used if the targeted agent is other than a peptide or protein, such a nucleic acid or a non-peptide drug. Any means known to those of skill in the art for chemically conjugating selected moieties may be used.

[0460] 2. Linkers

[0461] Linkers for two purposes are contemplated herein. The conjugates may include one or more linkers between the MTSP protein portion and the targeting agent. Additionally, linkers are used for facilitating or enhancing immobilization of an MTSP protein or portion thereof on a solid support, such as a microtiter plate, silicon or silicon-coated chip, glass or plastic support, such as for high throughput solid phase screening protocols.

[0462] Any linker known to those of skill in the art for preparation of conjugates may be used herein. These linkers are typically used in the preparation of chemical conjugates; peptide linkers may be incorporated into fusion proteins.

[0463] Linkers can be any moiety suitable to associate a domain of MTSP protein and a targeting agent. Such linkers and linkages include, but are not limited to, peptidic linkages, amino acid and peptide linkages, typically containing between one and about 60 amino acids, more generally between about 10 and 30 amino acids, chemical linkers, such as heterobifunctional cleavable cross-linkers, including but are not limited to, N-succinimidyl (4-iodoacetyl)-aminobenzoate, sulfosuccinimidyl (4-iodoacetyl)-aminobenzoate, 4-succinimidyl-oxycarbonyl-a-(2-pyridyldithio)toluene, sulfosuccinimidyl-6-[a-methyl-a-(pyridyldithio)toluamido]hexanoate, N-succinimidyl-3-(2-pyridyldithio)-propionate, succinimidyl 6[3-(2-pyridyldithio)-propionamido]hexanoate, sulfosuccinimidyl 6[3-(2-pyridyldithio)-propionamido]hexanoate, 3-(2-pyridyldithio)-propionyl hydrazide, Ellman's reagent, dichlorotriazinic acid, and S-(2-thiopyridyl)-L-cysteine. Other linkers include, but are not limited to peptides and other moieties that reduce steric hindrance between the domain of MTSP protein and the targeting agent, intracellular enzyme substrates, linkers that increase the flexibility of the conjugate, linkers that increase the solubility of the conjugate, linkers that increase the serum stability of the conjugate, photocleavable linkers and acid cleavable linkers.

[0464] Other exemplary linkers and linkages that are suitable for chemically linked conjugates include, but are not limited to, disulfide bonds, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups. These bonds are produced

using heterobifunctional reagents to produce reactive thiol groups on one or both of the polypeptides and then reacting the thiol groups on one polypeptide with reactive thiol groups or amine groups to which reactive maleimido groups or thiol groups can be attached on the other. Other linkers include, acid cleavable linkers, such as bismaleimidoethoxy propane, acid labile-transferrin conjugates and adipic acid dihydrazide, that would be cleaved in more acidic intracellular compartments; cross linkers that are cleaved upon exposure to UV or visible light and linkers, such as the various domains, such as C<sub>H</sub>1, C<sub>H</sub>2, and C<sub>H</sub>3, from the constant region of human IgG<sub>1</sub> (see, Batra et al. *Molecular Immunol.*, 30:379-386 (1993)). In some embodiments, several linkers may be included in order to take advantage of desired properties of each linker.

[0465] Chemical linkers and peptide linkers may be inserted by covalently coupling the linker to the domain of MTSP protein and the targeting agent. The heterobifunctional agents, described below, may be used to effect such covalent coupling. Peptide linkers may also be linked by expressing DNA encoding the linker and TA, linker and targeted agent, or linker, targeted agent and TA as a fusion protein. Flexible linkers and linkers that increase solubility of the conjugates are contemplated for use, either alone or with other linkers are also contemplated herein.

[0466] a) Acid Cleavable, Photocleavable and Heat Sensitive Linkers

[0467] Acid cleavable linkers, photocleavable and heat sensitive linkers may also be used, particularly where it may be necessary to cleave the domain of MTSP protein to permit it to be more readily accessible to reaction. Acid cleavable linkers include, but are not limited to, bismaleimidoethoxy propane; and adipic acid dihydrazide linkers (see, e.g., Fattom et al. (1992) *Infection & Immun.* 60:584-589) and acid labile transferrin conjugates that contain a sufficient portion of transferrin to permit entry into the intracellular transferrin cycling pathway (see, e.g., Welhöner et al. (1991) *J. Biol. Chem.* 266:4309-4314).

[0468] Photocleavable linkers are linkers that are cleaved upon exposure to light (see, e.g., Goldmacher et al. (1992) *Bioconj. Chem.* 3:104-107, which linkers are herein incorporated by reference), thereby releasing the targeted agent upon exposure to light. Photocleavable linkers that are cleaved upon exposure to light are known (see, e.g., Hazum et al. (1981) in *Pept., Proc. Eur. Pept. Symp.*, 16th, Brunfeldt, K (Ed), pp. 105-110, which describes the use of a nitrobenzyl group as a photocleavable protective group for cysteine; Yen et al. (1989) *Makromol. Chem* 190:69-82, which describes water soluble photocleavable copolymers, including hydroxypropylmethacrylamide copolymer, glycine copolymer, fluorescein copolymer and methylrhodamine copolymer; Goldmacher et al. (1992) *Bioconj. Chem.* 3:104-107, which describes a cross-linker and reagent that undergoes photolytic degradation upon exposure to near UV light (350 nm); and Senter et al. (1985) *Photochem. Photobiol* 42:231-237, which describes nitrobenzyloxycarbonyl chloride cross linking reagents that produce photocleavable linkages), thereby releasing the targeted agent upon exposure to light. Such linkers would have particular use in treating dermatological or ophthalmic conditions that can be exposed to light using fiber optics. After administration of the conjugate, the eye or skin or

other body part can be exposed to light, resulting in release of the targeted moiety from the conjugate. Such photocleavable linkers are useful in connection with diagnostic protocols in which it is desirable to remove the targeting agent to permit rapid clearance from the body of the animal.

**[0469]** b) Other Linkers for Chemical Conjugation

**[0470]** Other linkers, include trityl linkers, particularly, derivatized trityl groups to generate a genus of conjugates that provide for release of therapeutic agents at various degrees of acidity or alkalinity. The flexibility thus afforded by the ability to preselect the pH range at which the therapeutic agent will be released allows selection of a linker based on the known physiological differences between tissues in need of delivery of a therapeutic agent (see, e.g., U.S. Pat. No. 5,612,474). For example, the acidity of tumor tissues appears to be lower than that of normal tissues.

**[0471]** c) Peptide Linkers

**[0472]** The linker moieties can be peptides. Peptide linkers can be employed in fusion proteins and also in chemically linked conjugates. The peptide typically has from about 2 to about 60 amino acid residues, for example from about 5 to about 40, or from about 10 to about 30 amino acid residues. The length selected will depend upon factors, such as the use for which the linker is included.

**[0473]** Peptide linkers are advantageous when the targeting agent is proteinaceous. For example, the linker moiety can be a flexible spacer amino acid sequence, such as those known in single-chain antibody research. Examples of such known linker moieties include, but are not limited to, peptides, such as  $(\text{Gly}_m\text{Ser})_n$  and  $(\text{Ser}_m\text{Gly})_n$ , in which n is 1 to 6, preferably 1 to 4, more preferably 2 to 4, and m is 1 to 6, preferably 1 to 4, more preferably 2 to 4, enzyme cleavable linkers and others.

**[0474]** Additional linking moieties are described, for example, in Huston et al., *Proc. Natl. Acad. Sci. U.S.A.* 85:5879-5883, 1988; Whitlow, M., et al., *Protein Engineering* 6:989-995, 1993; Newton et al., *Biochemistry* 35:545-553, 1996; A. J. Cumber et al., *Bioconj. Chem.* 3:397-401, 1992; Ladurner et al., *J. Mol. Biol.* 273:330-337, 1997; and U.S. Pat. No. 4,894,443. In some embodiments, several linkers may be included in order to take advantage of desired properties of each linker.

**[0475]** 3. Targeting Agents

**[0476]** Any agent that facilitates detection, immobilization, or purification of the conjugate is contemplated for use herein. For chemical conjugates any moiety that has such properties is contemplated; for fusion proteins, the targeting agent is a protein, peptide or fragment thereof that sufficient to effects the targeting activity. Preferred targeting agents are those that deliver the MTSP protein or portion thereof to selected cells and tissues. Such agents include tumor specific monoclonal antibodies and portions thereof, growth factors, such as FGF, EGF, PDGF, VEGF, cytokines, including chemokines, and other such agents.

**[0477]** 4. Nucleic Acids, Plasmids and Cells

**[0478]** Isolated nucleic acid fragments encoding fusion proteins are provided. The nucleic acid fragment that encodes the fusion protein includes: a) nucleic acid encoding a protease domain of an MTSP protein encoded by a nucleic

acid that hybridizes to a nucleic acid having the nucleotide sequence set forth in the SEQ. ID NO:1; and b) nucleic acid encoding a protein, peptide or effective fragment thereof that facilitates: i) affinity isolation or purification of the fusion protein; ii) attachment of the fusion protein to a surface; or iii) detection of the fusion protein. Preferably, the nucleic acid is DNA.

**[0479]** Plasmids for replication and vectors for expression that contain the above nucleic acid fragments are also provided. Cells containing the plasmids and vectors are also provided. The cells can be any suitable host including, but are not limited to, bacterial cells, yeast cells, fungal cells, plant cells, insect cell and animal cells. The nucleic acids, plasmids, and cells containing the plasmids can be prepared according to methods known in the art including any described herein.

**[0480]** Also provided are methods for producing the above fusion proteins. An exemplary method includes the steps of growing, i.e. culturing the cells so that the proliferate, cells containing a plasmid encoding the fusion protein under conditions whereby the fusion protein is expressed by the cell, and recovering the expressed fusion protein. Methods for expressing and recovering recombinant proteins are well known in the art (See generally, *Current Protocols in Molecular Biology* (1998) § 16, John Wiley & Sons, Inc.) and such methods can be used for expressing and recovering the expressed fusion proteins. Preferably, the recombinant expression and recovery methods disclosed in Section B can be used.

**[0481]** The recovered fusion proteins can be isolated or purified by methods known in the art such as centrifugation, filtration, chromatograph, electrophoresis, immunoprecipitation, etc., or by a combination thereof (See generally, *Current Protocols in Molecular Biology* (1998) § 10, John Wiley & Sons, Inc.). Preferably, the recovered fusion protein is isolated or purified through affinity binding between the protein or peptide fragment of the fusion protein and an affinity binding moiety. As discussed in the above sections regarding the construction of the fusion proteins, any affinity binding pairs can be constructed and used in the isolation or purification of the fusion proteins. For example, the affinity binding pairs can be protein binding sequences/protein, DNA binding sequences/DNA sequences, RNA binding sequences/RNA sequences, lipid binding sequences/lipid, polysaccharide binding sequences/polysaccharide, or metal binding sequences/metal.

**[0482]** 5. Immobilization and Supports or Substrates Therefor

**[0483]** In certain embodiments, where the targeting agents are designed for linkage to surfaces, the MTSP protein can be attached by linkage such as ionic or covalent, non-covalent or other chemical interaction, to a surface of a support or matrix material. Immobilization may be effected directly or via a linker. The MTSP protein may be immobilized on any suitable support, including, but are not limited to, silicon chips, and other supports described herein and known to those of skill in the art. A plurality of MTSP protein or protease domains thereof may be attached to a support, such as an array (i.e., a pattern of two or more) of conjugates on the surface of a silicon chip or other chip for use in high throughput protocols and formats.

**[0484]** It is also noted that the domains of the MTSP protein can be linked directly to the surface or via a linker

without a targeting agent linked thereto. Hence chips containing arrays of the domains of the MTSP protein.

[0485] The matrix material or solid supports contemplated herein are generally any of the insoluble materials known to those of skill in the art to immobilize ligands and other molecules, and are those that used in many chemical syntheses and separations. Such supports are used, for example, in affinity chromatography, in the immobilization of biologically active materials, and during chemical syntheses of biomolecules, including proteins, amino acids and other organic molecules and polymers. The preparation of and use of supports is well known to those of skill in this art; there are many such materials and preparations thereof known. For example, naturally-occurring support materials, such as agarose and cellulose, may be isolated from their respective sources, and processed according to known protocols, and synthetic materials may be prepared in accord with known protocols.

[0486] The supports are typically insoluble materials that are solid, porous, deformable, or hard, and have any required structure and geometry, including, but not limited to: beads, pellets, disks, capillaries, hollow fibers, needles, solid fibers, random shapes, thin films and membranes. Thus, the item may be fabricated from the matrix material or combined with it, such as by coating all or part of the surface or impregnating particles.

[0487] Typically, when the matrix is particulate, the particles are at least about 10-2000  $\mu\text{M}$ , but may be smaller or larger, depending upon the selected application. Selection of the matrices will be governed, at least in part, by their physical and chemical properties, such as solubility, functional groups, mechanical stability, surface area swelling propensity, hydrophobic or hydrophilic properties and intended use.

[0488] If necessary, the support matrix material can be treated to contain an appropriate reactive moiety. In some cases, the support matrix material already containing the reactive moiety may be obtained commercially. The support matrix material containing the reactive moiety may thereby serve as the matrix support upon which molecules are linked. Materials containing reactive surface moieties such as amino silane linkages, hydroxyl linkages or carboxysilane linkages may be produced by well established surface chemistry techniques involving silanization reactions, or the like. Examples of these materials are those having surface silicon oxide moieties, covalently linked to gamma-amino-propylsilane, and other organic moieties; N-[3-(triethoxysilyl)propyl]-phthelamic acid; and bis-(2-hydroxyethyl)amino-propyltriethoxysilane. Exemplary of readily available materials containing amino group reactive functionalities, include, but are not limited to, para-aminophenyl-triethoxysilane. Also derivatized polystyrenes and other such polymers are well known and readily available to those of skill in this art (e.g., the Tentagel® Resins are available with a multitude of functional groups, and are sold by Rapp Polymere, Tübingen, Germany; see, U.S. Pat. No. 4,908,405 and U.S. Pat. No. 5,292,814; see, also Butz et al., *Peptide Res.*, 7:20-23 (1994); and Kleine et al., *Immunobiol.*, 190:53-66 (1994)).

[0489] These matrix materials include any material that can act as a support matrix for attachment of the molecules of interest. Such materials are known to those of skill in this

art, and include those that are used as a support matrix. These materials include, but are not limited to, inorganics, natural polymers, and synthetic polymers, including, but are not limited to: cellulose, cellulose derivatives, acrylic resins, glass, silica gels, polystyrene, gelatin, polyvinyl pyrrolidone, co-polymers of vinyl and acrylamide, polystyrene cross-linked with divinylbenzene and others (see, Merrifield, *Biochemistry*, 3:1385-1390 (1964)), polyacrylamides, latex gels, polystyrene, dextran, polyacrylamides, rubber, silicon, plastics, nitrocellulose, celluloses, natural sponges. Of particular interest herein, are highly porous glasses (see, e.g., U.S. Pat. No. 4,244,721) and others prepared by mixing a borosilicate, alcohol and water.

[0490] Synthetic supports include, but are not limited to: acrylamides, dextran-derivatives and dextran co-polymers, agarose-polyacrylamide blends, other polymers and co-polymers with various functional groups, methacrylate derivatives and co-polymers, polystyrene and polystyrene copolymers (see, e.g., Merrifield, *Biochemistry*, 3:1385-1390 (1964); Berg et al., in *Innovation Perspect. Solid Phase Synth. Collect. Pap.*, Int. Symp., 1st, Epton, Roger (Ed), pp. 453-459 (1990); Berg et al., *Pept., Proc. Eur. Pept. Symp.*, 20th, Jung, G. et al. (Eds), pp. 196-198 (1989); Berg et al., *J. Am. Chem. Soc.*, 111:8024-8026 (1989); Kent et al., *Isr. J. Chem.*, 17:243-247 (1979); Kent et al., *J. Org. Chem.*, 43:2845-2852 (1978); Mitchell et al., *Tetrahedron Lett.*, 42:3795-3798 (1976); U.S. Pat. No. 4,507,230; U.S. Pat. No. 4,006,117; and U.S. Pat. No. 5,389,449). Such materials include those made from polymers and co-polymers such as polyvinylalcohols, acrylates and acrylic acids such as polyethylene-co-acrylic acid, polyethylene-co-methacrylic acid, polyethylene-co-ethylacrylate, polyethylene-co-methyl acrylate, polypropylene-co-acrylic acid, polypropylene-co-methyl-acrylic acid, polypropylene-co-ethylacrylate, polypropylene-co-methyl acrylate, polyethylene-co-vinyl acetate, polypropylene-co-vinyl acetate, and those containing acid anhydride groups such as polyethylene-co-maleic anhydride and polypropylene-co-maleic anhydride. Liposomes have also been used as solid supports for affinity purifications (Powell et al. *Biotechnol. Bioeng.*, 33:173 (1989)).

[0491] Numerous methods have been developed for the immobilization of proteins and other biomolecules onto solid or liquid supports (see, e.g., Mosbach, *Methods in Enzymology*, 44 (1976); Weetall, *Immobilized Enzymes, Antigens, Antibodies, and Peptides*, (1975); Kennedy et al., *Solid Phase Biochemistry, Analytical and Synthetic Aspects*, Scouten, ed., pp. 253-391 (1983); see, generally, Affinity Techniques. Enzyme Purification: *Part B. Methods in Enzymology*, Vol. 34, ed. W. B. Jakoby, M. Wilchek, Acad. Press, N.Y. (1974); and Immobilized Biochemicals and Affinity Chromatography, *Advances in Experimental Medicine and Biology*, vol. 42, ed. R. Dunlap, Plenum Press, N.Y. (1974)).

[0492] Among the most commonly used methods are absorption and adsorption or covalent binding to the support, either directly or via a linker, such as the numerous disulfide linkages, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups, known to those of skill in art (see, e.g., the PIERCE CATALOG, ImmunoTechnology Catalog & Handbook, 1992-1993, which describes the preparation of and use of such reagents and provides a commercial source for such reagents; Wong, *Chemistry of Protein Conjugation*

and Cross Linking, CRC Press (1993); see also DeWitt et al., *Proc. Natl. Acad. Sci. U.S.A.*, 90:6909 (1993); Zuckermann et al., *J. Am. Chem. Soc.*, 114:10646 (1992); Kurth et al., *J. Am. Chem. Soc.*, 116:2661 (1994); Ellman et al., *Proc. Natl. Acad. Sci. U.S.A.*, 91:4708 (1994); Sucholeiki, *Tetrahedron Lett.*, 35:7307 (1994); Su-Sun Wang, *J. Org. Chem.*, 41:3258 (1976); Padwa et al., *J. Org. Chem.*, 41:3550 (1971); and Vedejs et al., *J. Org. Chem.*, 49:575 (1984), which describe photosensitive linkers).

[0493] To effect immobilization, a composition containing the protein or other biomolecule is contacted with a support material such as alumina, carbon, an ion-exchange resin, cellulose, glass or a ceramic. Fluorocarbon polymers have been used as supports to which biomolecules have been attached by adsorption (see, U.S. Pat. No. 3,843,443; Published International PCT Application WO/86 03840).

[0494] J. Prognosis and Diagnosis

[0495] MTSP protein proteins, domains, analogs, and derivatives thereof, and encoding nucleic acids (and sequences complementary thereto), and anti-MTSP protein antibodies, can be used in diagnostics. Such molecules can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting MTSP protein expression, or monitor the treatment thereof. For purposes herein, the presence of MTSPs in body fluids or tumor tissues are of particular interest.

[0496] In particular, such an immunoassay is carried out by a method including contacting a sample derived from a patient with an anti-MTSP protein antibody under conditions such that specific binding can occur, and detecting or measuring the amount of any specific binding by the antibody. In a specific aspect, such binding of antibody, in tissue sections, can be used to detect aberrant MTSP protein localization or aberrant (e.g., low or absent) levels of MTSP protein. In a specific embodiment, antibody to MTSP protein can be used to assay in a patient tissue or serum sample for the presence of MTSP protein where an aberrant level of MTSP protein is an indication of a diseased condition.

[0497] The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few.

[0498] MTSP protein genes and related nucleic acid sequences and subsequences, including complementary sequences, can also be used in hybridization assays. MTSP protein nucleic acid sequences, or subsequences thereof containing about at least 8 nucleotides, preferably 14 or 16 or 30 or more contiguous nucleotides, can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant changes in MTSP protein expression and/or activity as described herein. In particular, such a hybridization assay is carried out by a method by contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to MTSP protein

encoding DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

[0499] In a specific embodiment, a method of diagnosing a disease or disorder characterized by detecting an aberrant level of an MTSP protein in a subject is provided herein by measuring the level of the DNA, RNA, protein or functional activity of the epithelial MTSP protein at least partially encoded by a nucleic acid that hybridizes to a nucleic acid having the nucleotide sequence set forth in the SEQ. ID NO:1 in a sample derived from the subject, wherein an increase or decrease in the level of the DNA, RNA, protein or functional activity of the MTSP protein, relative to the level of the DNA, RNA, protein or functional activity found in an analogous sample not having the disease or disorder indicates the presence of the disease or disorder in the subject.

[0500] Kits for diagnostic use are also provided, that contain in one or more containers an anti-MTSP protein antibody, particularly anti-MTSP3 or anti-MTSP4, and, optionally, a labeled binding partner to the antibody. Alternatively, the anti-MTSP protein antibody can be labeled (with a detectable marker, e.g., a chemiluminescent, enzymatic, fluorescent, or radioactive moiety). A kit is also provided that includes in one or more containers a nucleic acid probe capable of hybridizing to MTSP protein-encoding RNA. In a specific embodiment, a kit can comprise in one or more containers a pair of primers (e.g., each in the size range of 6-30 nucleotides) that are capable of priming amplification [e.g., by polymerase chain reaction (see e.g., Innis et al., 1990, PCR Protocols, Academic Press, Inc., San Diego, Calif.), ligase chain reaction (see EP 320,308) use of Q $\beta$  replicase, cyclic probe reaction, or other methods known in the art under appropriate reaction conditions of at least a portion of an MTSP protein-encoding nucleic acid. A kit can optionally further comprise in a container a predetermined amount of a purified MTSP protein or nucleic acid, e.g., for use as a standard or control.

[0501] K. Pharmaceutical Compositions and Modes of Administration

[0502] 1. Components of the Compositions

[0503] Pharmaceutical compositions containing the identified compounds that modulate the activity of an MTSP protein are provided herein. Also provided are combinations of a compound that modulates the activity of an MTSP protein and another treatment or compound for treatment of a neoplastic disorder, such as a chemotherapeutic compound.

[0504] The MTSP protein modulator and the anti-tumor agent can be packaged as separate compositions for administration together or sequentially or intermittently. Alternatively, they can be provided as a single composition for administration or as two compositions for administration as a single composition. The combinations can be packaged as kits.

[0505] a. MTSP Protein Inhibitors

[0506] Any MTSP protein inhibitors, including those described herein when used alone or in combination with other compounds, that can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of

clinical symptoms or diagnostic markers associated with neoplastic diseases, including undesired and/or uncontrolled angiogenesis, can be used in the present combinations.

**[0507]** In one embodiment, the MTSP protein inhibitor is an antibody or fragment thereof that specifically reacts with an MTSP protein or the protease domain thereof, an inhibitor of the MTSP protein production, an inhibitor of the epithelial MTSP protein membrane-localization, or any inhibitor of the expression of or, especially, the activity of an MTSP protein.

**[0508]** b. Anti-angiogenic Agents and Anti-tumor Agents

**[0509]** Any anti-angiogenic agents and anti-tumor agents, including those described herein, when used alone or in combination with other compounds, that can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with undesired and/or uncontrolled angiogenesis and/or tumor growth and metastasis, particularly solid neoplasms, vascular malformations and cardiovascular disorders, chronic inflammatory diseases and aberrant wound repairs, circulatory disorders, crest syndromes, dermatological disorders, or ocular disorders, can be used in the combinations. Also contemplated are anti-tumor agents for use in combination with an inhibitor of an MTSP protein.

**[0510]** C. Anti-tumor Agents and Anti-angiogenic Agents

**[0511]** The compounds identified by the methods provided herein or provided herein can be used in combination with anti-tumor agents and/or anti-angiogenesis agents.

**[0512]** 2. Formulations and Route of Administration

**[0513]** The compounds herein and agents are preferably formulated as pharmaceutical compositions, preferably for single dosage administration. The concentrations of the compounds in the formulations are effective for delivery of an amount, upon administration, that is effective for the intended treatment. Typically, the compositions are formulated for single dosage administration. To formulate a composition, the weight fraction of a compound or mixture thereof is dissolved, suspended, dispersed or otherwise mixed in a selected vehicle at an effective concentration such that the treated condition is relieved or ameliorated. Pharmaceutical carriers or vehicles suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration.

**[0514]** In addition, the compounds may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients. Liposomal suspensions, including tissue-targeted liposomes, may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be prepared as described in U.S. Pat. No. 4,522,811.

**[0515]** The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the compounds in known *in vitro* and *in vivo* systems, such as the assays provided herein.

**[0516]** The concentration of active compound in the drug composition will depend on absorption, inactivation and excretion rates of the active compound, the physicochemical characteristics of the compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

**[0517]** Typically a therapeutically effective dosage is contemplated. The amounts administered may be on the order of 0.001 to 1 mg/ml, preferably about 0.005-0.05 mg/ml, more preferably about 0.01 mg/ml, of blood volume. Pharmaceutical dosage unit forms are prepared to provide from about 1 mg to about 1000 mg and preferably from about 10 to about 500 mg, more preferably about 25-75 mg of the essential active ingredient or a combination of essential ingredients per dosage unit form. The precise dosage can be empirically determined.

**[0518]** The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data. It is to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or use of the claimed compositions and combinations containing them.

**[0519]** Preferred pharmaceutically acceptable derivatives include acids, salts, esters, hydrates, solvates and prodrug forms. The derivative is typically selected such that its pharmacokinetic properties are superior to the corresponding neutral compound.

**[0520]** Thus, effective concentrations or amounts of one or more of the compounds provided herein or pharmaceutically acceptable derivatives thereof are mixed with a suitable pharmaceutical carrier or vehicle for systemic, topical or local administration to form pharmaceutical compositions. Compounds are included in an amount effective for ameliorating or treating the disorder for which treatment is contemplated. The concentration of active compound in the composition will depend on absorption, inactivation, excretion rates of the active compound, the dosage schedule, amount administered, particular formulation as well as other factors known to those of skill in the art.

**[0521]** Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any of the following components: a sterile diluent, such as water for injection, saline solution, fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents, such as benzyl alcohol and methyl parabens; antioxidants, such as ascorbic acid and sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates and phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. Parenteral preparations can be enclosed in ampules, disposable syringes or single or multiple dose vials made of glass, plastic or other suitable material.

[0522] In instances in which the compounds exhibit insufficient solubility, methods for solubilizing compounds may be used. Such methods are known to those of skill in this art, and include, but are not limited to, using cosolvents, such as dimethylsulfoxide (DMSO), using surfactants, such as Tween®, or dissolution in aqueous sodium bicarbonate. Derivatives of the compounds, such as prodrugs of the compounds may also be used in formulating effective pharmaceutical compositions. For ophthalmic indications, the compositions are formulated in an ophthalmically acceptable carrier. For the ophthalmic uses herein, local administration, either by topical administration or by injection is preferred. Time release formulations are also desirable. Typically, the compositions are formulated for single dosage administration, so that a single dose administers an effective amount.

[0523] Upon mixing or addition of the compound with the vehicle, the resulting mixture may be a solution, suspension, emulsion or other composition. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. If necessary, pharmaceutically acceptable salts or other derivatives of the compounds are prepared.

[0524] The compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. It is understood that number and degree of side effects depends upon the condition for which the compounds are administered. For example, certain toxic and undesirable side effects are tolerated when treating life-threatening illnesses that would not be tolerated when treating disorders of lesser consequence.

[0525] The compounds can also be mixed with other active materials, that do not impair the desired action, or with materials that supplement the desired action known to those of skill in the art. The formulations of the compounds and agents for use herein include those suitable for oral, rectal, topical, inhalational, buccal (e.g., sublingual), parenteral (e.g., subcutaneous, intramuscular, intradermal, or intravenous), transdermal administration or any route. The most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular active compound which is being used. The formulations are provided for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil-water emulsions containing suitable quantities of the compounds or pharmaceutically acceptable derivatives thereof. The pharmaceutically therapeutically active compounds and derivatives thereof are typically formulated and administered in unit-dosage forms or multiple-dosage forms. Unit-dose forms as used herein refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of the therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit-dose forms include ampoules and syringes and individually packaged tablets or capsules. Unit-dose forms may be administered in fractions or multiples thereof. A multiple-dose form is a plurality of identical

unit-dosage forms packaged in a single container to be administered in segregated unit-dose form. Examples of multiple-dose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit-doses which are not segregated in packaging.

[0526] The composition can contain along with the active ingredient: a diluent such as lactose, sucrose, dicalcium phosphate, or carboxymethyl-cellulose; a lubricant, such as magnesium stearate, calcium stearate and talc; and a binder such as starch, natural gums, such as gum acaciagelatin, glucose, molasses, polyvinylpyrrolidone, celluloses and derivatives thereof, povidone, crospovidones and other such binders known to those of skill in the art. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, or otherwise mixing an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, or solubilizing agents, pH buffering agents and the like, for example, acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents. Methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art (see, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 15th Edition, 1975). The composition or formulation to be administered will contain a quantity of the active compound in an amount sufficient to alleviate the symptoms of the treated subject.

[0527] Dosage forms or compositions containing active ingredient in the range of 0.005% to 100% with the balance made up from non-toxic carrier may be prepared. For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art.

[0528] The pharmaceutical preparation may also be in liquid form, for example, solutions, syrups or suspensions, or may be presented as a drug product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid).

[0529] Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the active compound with one or

more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

**[0530]** Formulations suitable for topical application to the skin or to the eye preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol and oil. Carriers which may be used include vaseline, lanoline, polyethylene glycols, alcohols, and combinations of two or more thereof. The topical formulations may further advantageously contain 0.05 to 15 percent by weight of thickeners selected from among hydroxypropyl methyl cellulose, methyl cellulose, polyvinylpyrrolidone, polyvinyl alcohol, poly (alkylene glycols), poly/hydroxyalkyl, (meth)acrylates or poly(meth)acrylamides. A topical formulation is often applied by instillation or as an ointment into the conjunctival sac. It can also be used for irrigation or lubrication of the eye, facial sinuses, and external auditory meatus. It may also be injected into the anterior eye chamber and other places. The topical formulations in the liquid state may be also present in a hydrophilic three-dimensional polymer matrix in the form of a strip, contact lens, and the like from which the active components are released.

**[0531]** For administration by inhalation, the compounds for use herein can be delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin, for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

**[0532]** Formulations suitable for buccal (sublingual) administration include, for example, lozenges containing the active compound in a flavored base, usually sucrose and acacia or tragacanth; and pastilles containing the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

**[0533]** The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampules or in multi-dose containers, with an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for reconstitution with a suitable vehicle, e.g., sterile pyrogen-free water or other solvents, before use.

**[0534]** Formulations suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound as an optionally buffered aqueous solution of, for example, 0.1 to 0.2 M concentration with respect to the active compound. Formulations suitable for transdermal administration may also be delivered by iontophoresis (see, e.g., *Pharmaceutical Research* 3 (6), 318 (1986)) and typically take the form of an optionally buffered aqueous solution of the active compound.

**[0535]** The pharmaceutical compositions may also be administered by controlled release means and/or delivery

devices (see, e.g., in U.S. Pat. Nos. 3,536,809; 3,598,123; 3,630,200; 3,845,770; 3,847,770; 3,916,899; 4,008,719; 4,687,610; 4,769,027; 5,059,595; 5,073,543; 5,120,548; 5,354,566; 5,591,767; 5,639,476; 5,674,533 and 5,733,566).

**[0536]** Desirable blood levels may be maintained by a continuous infusion of the active agent as ascertained by plasma levels. It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust therapy to lower dosage due to toxicity, or bone marrow, liver or kidney dysfunctions. Conversely, the attending physician would also know how to and when to adjust treatment to higher levels if the clinical response is not adequate (precluding toxic side effects).

**[0537]** The efficacy and/or toxicity of the MTSP protein inhibitor(s), alone or in combination with other agents can also be assessed by the methods known in the art (See generally, O'Reilly, *Investigational New Drugs*, 15:5-13 (1997)).

**[0538]** The active compounds or pharmaceutically acceptable derivatives may be prepared with carriers that protect the compound against rapid elimination from the body, such as time release formulations or coatings.

**[0539]** Kits containing the compositions and/or the combinations with instructions for administration thereof are provided. The kit may further include a needle or syringe, preferably packaged in sterile form, for injecting the complex, and/or a packaged alcohol pad. Instructions are optionally included for administration of the active agent by a clinician or by the patient.

**[0540]** Finally, the compounds or MTSP proteins or protease domains thereof or compositions containing any of the preceding agents may be packaged as articles of manufacture containing packaging material, a compound or suitable derivative thereof provided herein, which is effective for treatment of a diseases or disorders contemplated herein, within the packaging material, and a label that indicates that the compound or a suitable derivative thereof is for treating the diseases or disorders contemplated herein. The label can optionally include the disorders for which the therapy is warranted.

**[0541]** L. Methods of Treatment

**[0542]** The compounds identified by the methods herein are used for treating or preventing neoplastic diseases in an animal, particularly a mammal, including a human, is provided herein. In one embodiment, the method includes administering to a mammal an effective amount of an inhibitor of an MTSP protein, whereby the disease or disorder is treated or prevented. In a preferred embodiment, the MTSP protein inhibitor used in the treatment or prevention is administered with a pharmaceutically acceptable carrier or excipient. The mammal treated can be a human. The inhibitors provided herein are those identified by the screening assays. In addition, antibodies and antisense nucleic acids are contemplated.

**[0543]** The treatment or prevention method can further include administering an anti-angiogenic treatment or agent or anti-tumor agent simultaneously with, prior to or subsequent to the MTSP protein inhibitor, which can be any compound identified that inhibits the activity of an MTSP protein, and includes an antibody or a fragment or derivative

thereof containing the binding region thereof against the MTSP protein, an antisense nucleic acid encoding the MTSP protein, and a nucleic acid containing at least a portion of a gene encoding the MTSP protein into which a heterologous nucleotide sequence has been inserted such that the heterologous sequence inactivates the biological activity of at least a portion of the gene encoding the MTSP protein, in which the portion of the gene encoding the MTSP protein flanks the heterologous sequence so as to promote homologous recombination with a genomic gene encoding the MTSP protein.

**[0544]** 1. Antisense Treatment

**[0545]** In a specific embodiment, as described hereinabove, MTSP protein function is reduced or inhibited by MTSP protein antisense nucleic acids, to treat or prevent neoplastic disease. The therapeutic or prophylactic use of nucleic acids of at least six nucleotides that are antisense to a gene or cDNA encoding MTSP protein or a portion thereof. An MTSP protein "antisense" nucleic acid as used herein refers to a nucleic acid capable of hybridizing to a portion of an MTSP protein RNA (preferably mRNA) by virtue of some sequence complementarity. The antisense nucleic acid may be complementary to a coding and/or noncoding region of an MTSP protein mRNA. Such antisense nucleic acids have utility as therapeutics that reduce or inhibit MTSP protein function, and can be used in the treatment or prevention of disorders as described supra.

**[0546]** The MTSP protein antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 150 nucleotides, or more preferably 6 to 50 nucleotides). In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 125 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci. U.S.A.* 84:648-652 (1987); PCT Publication No. WO 88/09810, published Dec. 15, 1988) or blood-brain barrier (see, e.g., PCT Publication No. WO 89/10134, published Apr. 25, 1988), hybridization-triggered cleavage agents (see, e.g., Krol et al., *BioTechniques* 6:958-976 (1988)) or intercalating agents (see, e.g., Zon, *Pharm. Res.* 5:539-549 (1988)).

**[0547]** The MTSP protein antisense nucleic acid is preferably an oligonucleotide, more preferably of single-stranded DNA. In a preferred aspect, the oligonucleotide includes a sequence antisense to a portion of human MTSP protein. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

**[0548]** The MTSP protein antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethyl-aminomethyluracil, dihydrouracil, beta-D-ga-

lactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl)uracil, (acp3)w, and 2,6-diaminopurine.

**[0549]** In another embodiment, the oligonucleotide includes at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose. The oligonucleotide can include at least one modified phosphate backbone selected from a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

**[0550]** The oligonucleotide can be an  $\alpha$ -anomeric oligonucleotide. An  $\alpha$ -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which the strands run parallel to each other (Gautier et al., *Nucl. Acids Res.* 15:6625-6641 (1987)).

**[0551]** The oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent and hybridization-triggered cleavage agent.

**[0552]** The oligonucleotides may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (*Nucl. Acids Res.* 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., *Proc. Natl. Acad. Sci. U.S.A.* 85:7448-7451 (1988)), etc.

**[0553]** In a specific embodiment, the MTSP protein antisense oligonucleotide includes catalytic RNA, or a ribozyme (see, e.g., PCT International Publication WO 90/11364, published Oct. 4, 1990; Sarver et al., *Science* 247:1222-1225 (1990)). In another embodiment, the oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., *Nucl. Acids Res.* 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., *FEBS Lett.* 215:327-330 (1987)).

**[0554]** In an alternative embodiment, the MTSP protein antisense nucleic acid is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced in vivo such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA). Such a vector would contain a sequence encoding the MTSP protein antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replica-

tion and expression in mammalian cells. Expression of the sequence encoding the MTSP protein antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, *Nature* 290:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980), the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39-42 (1982), etc.

[0555] The antisense nucleic acids include sequence complementary to at least a portion of an RNA transcript of an MTSP protein gene, preferably a human MTSP protein gene. Absolute complementarity, although preferred, is not required.

[0556] The amount of MTSP protein antisense nucleic acid that will be effective in the treatment or prevention of neoplastic disease will depend on the nature of the disease, and can be determined empirically by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity in cells in vitro, and then in useful animal model systems prior to testing and use in humans.

[0557] 2. Gene Therapy

[0558] In an exemplary embodiment, nucleic acids that include a sequence of nucleotides encoding an MTSP protein or functional domains or derivative thereof, are administered to promote MTSP protein function, by way of gene therapy. Gene therapy refers to therapy performed by the administration of a nucleic acid to a subject. In this embodiment, the nucleic acid produces its encoded protein that mediates a therapeutic effect by promoting MTSP protein function. Any of the methods for gene therapy available in the art can be used (see, Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *An. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *An. Rev. Biochem.* 62:191-217 (1993); *TIBTECH* 11(5):155-215 (1993). For example, one therapeutic composition for gene therapy includes an MTSP protein-encoding nucleic acid that is part of an expression vector that expresses an MTSP protein or domain, fragment or chimeric protein thereof in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the MTSP protein coding region, the promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, a nucleic acid molecule is used in which the MTSP protein coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the MTSP protein nucleic acid (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989)).

[0559] Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector, or indirect, in which case, cells are first transformed with the nucleic acid in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

[0560] In a specific embodiment, the nucleic acid is directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by infection using a defective or attenuated retroviral or other viral vector (see U.S. Pat. No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand is a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180 dated Apr. 16, 1992 (Wu et al.); WO 92/22635 dated Dec. 23, 1992 (Wilson et al.); WO92/20316 dated Nov. 26, 1992 (Findeis et al.); WO93/14188 dated Jul. 22, 1993 (Clarke et al.); WO 93/20221 dated Oct. 14, 1993 (Young)). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989)).

[0561] In a specific embodiment, a viral vector that contains the MTSP protein nucleic acid is used. For example, a retroviral vector can be used (see Miller et al., *Meth. Enzymol.* 217:581-599 (1993)). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The MTSP protein nucleic acid to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdrl gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

[0562] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to trans-

fer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); and Mstrangeli et al., *J. Clin. Invest.* 91:225-234 (1993).

[0563] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993)).

[0564] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0565] In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92 (1985)) and may be used, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[0566] The resulting recombinant cells can be delivered to a patient by various methods known in the art. In a preferred embodiment, epithelial cells are injected, e.g., subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the patient. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0567] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0568] In a preferred embodiment, the cell used for gene therapy is autologous to the patient. In an embodiment in which recombinant cells are used in gene therapy, an MTSP protein nucleic acid is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated

and maintained in vitro can potentially be used in accordance with this embodiment. Such stem cells include but are not limited to hematopoietic stem cells (HSC), stem cells of epithelial tissues such as the skin and the lining of the gut, embryonic heart muscle cells, liver stem cells (PCT Publication WO 94/08598, dated Apr. 28, 1994), and neural stem cells (Stemple and Anderson, *Cell* 71:973-985 (1992)).

[0569] Epithelial stem cells (ESCs) or keratinocytes can be obtained from tissues such as the skin and the lining of the gut by known procedures (Rheinwald, *Meth. Cell Bio.* 21A:229 (1980)). In stratified epithelial tissue such as the skin, renewal occurs by mitosis of stem cells within the germinal layer, the layer closest to the basal lamina. Stem cells within the lining of the gut provide for a rapid renewal rate of this tissue. ESCs or keratinocytes obtained from the skin or lining of the gut of a patient or donor can be grown in tissue culture (Rheinwald, *Meth. Cell Bio.* 21A:229 (1980); Pittelkow and Scott, *Mayo Clinic Proc.* 61:771 (1986)). If the ESCs are provided by a donor, a method for suppression of host versus graft reactivity (e.g., irradiation, drug or antibody administration to promote moderate immunosuppression) can also be used.

[0570] With respect to hematopoietic stem cells (HSC), any technique which provides for the isolation, propagation, and maintenance in vitro of HSC can be used in this embodiment. Techniques by which this may be accomplished include (a) the isolation and establishment of HSC cultures from bone marrow cells isolated from the future host, or a donor, or (b) the use of previously established long-term HSC cultures, which may be allogeneic or xenogeneic. Non-autologous HSC are used preferably in conjunction with a method of suppressing transplantation immune reactions of the future host/patient. In a particular embodiment, human bone marrow cells can be obtained from the posterior iliac crest by needle aspiration (see, e.g., Kodo et al., *J. Clin. Invest.* 73:1377-1384 (1984)). In a preferred embodiment, the HSCs can be made highly enriched or in substantially pure form. This enrichment can be accomplished before, during, or after long-term culturing, and can be done by any techniques known in the art. Long-term cultures of bone marrow cells can be established and maintained by using, for example, modified Dexter cell culture techniques (Dexter et al., *J. Cell Physiol.* 91:335 (1977) or Witlock-Witte culture techniques (Witlock and Witte, *Proc. Natl. Acad. Sci. USA* 79:3608-3612 (1982)).

[0571] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy includes an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

[0572] 3. Prodrugs

[0573] A method for treating tumors is provided. The method is practiced by administering a prodrug that is specifically cleaved by an MTSP to release an active drug. Upon contact with a cell that expresses MTSP activity, the prodrug is converted into an active drug. The prodrug can be a conjugate that contains the active agent, such as an anti-tumor drug, such as a cytotoxic agent, or other atherapeutic agent, linked, linked to a substrate for the targeted MTSP, such that the drug or agent is inactive or unable to enter a cell, in the conjugate, but is activated upon cleavage.

The prodrug, for example, can contain an oligopeptide, preferably a relatively short, less than about 10 amino acids peptide, that is selectively proteolytically cleaved by the targeted MTSP. Cytotoxic agents, include, but are not limited to, alkylating agents, antiproliferative agents and tubulin binding agents. Others include, vinca drugs, mitomycins, bleomycins and taxanes.

#### [0574] M. Animal Models

[0575] Transgenic animal models are provided herein. Such an animal can be initially produced by promoting homologous recombination between an MTSP protein gene in its chromosome and an exogenous MTSP protein gene that has been rendered biologically inactive (preferably by insertion of a heterologous sequence, e.g., an antibiotic resistance gene). In a preferred aspect, this homologous recombination is carried out by transforming embryo-derived stem (ES) cells with a vector containing the insertionally inactivated MTSP protein gene, such that homologous recombination occurs, followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, followed by the birth of the chimeric animal ("knockout animal") in which an MTSP protein gene has been inactivated (see Capecchi, *Science* 244:1288-1292 (1989)). The chimeric animal can be bred to produce additional knockout animals. Such animals can be mice, hamsters, sheep, pigs, cattle, etc., and are preferably non-human mammals. In a specific embodiment, a knockout mouse is produced.

[0576] Such knockout animals are expected to develop or be predisposed to developing neoplastic diseases and thus can have use as animal models of such diseases e.g., to screen for or test molecules for the ability to treat or prevent such diseases or disorders. Hence, the animal models for are provided. Such an animal can be initially produced by promoting homologous recombination between an MTSP gene in its chromosome and an exogenous MTSP protein gene that would be over-expressed or mis-expressed (preferably by expression under a strong promoter). In a preferred aspect, this homologous recombination is carried out by transforming embryo-derived stem (ES) cells with a vector containing the over-expressed or mis-expressed MTSP protein gene, such that homologous recombination occurs, followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, followed by the birth of the chimeric animal in which an MTSP gene has been over-expressed or mis-expressed (see Capecchi, *Science* 244:1288-1292 (1989)). The chimeric animal can be bred to produce additional animals with over-expressed or mis-expressed MTSP protein. Such animals can be mice, hamsters, sheep, pigs, cattle, etc., and are preferably non-human mammals. In a specific embodiment, a mouse with over-expressed or mis-expressed MTSP protein is produced.

[0577] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

#### EXAMPLE 1

[0578] Cloning of MTSP3, Cloning and Mutagenesis of the Protease Domain of MTSP3

##### [0579] 1. Identification and Cloning of MTSP3

[0580] a. Identification of EST Clones AI924527 and AI924182 as Part of a Serine Protease MTSP3

[0581] DNA encoding the protease domain of the protease designated MTSP1 was independently cloned from the human prostatic adenocarcinoma cell line, PC-3, using degenerate oligonucleotide primers, then sequenced and characterized (see EXAMPLE 6). The sequence of the sense degenerate primer used in cloning MTSP1 was 5'-TGGR-T(I)VT(I)WS(I)GC(I)RC(I)CAYTG-3' (SEQ ID No: 13), and that of the anti-sense was 5'-(I)GG(I)CC(I)C-C(I)SWRTC(I)CCYT(I)RCA(I)GHRTC-3' (SEQ ID No:14), where R=A,G; V=G,A,C; W=A,T; S=G,C; Y=C,T; H=A,T,C. The primer sequences correspond to two highly conserved regions in all serine proteases and should amplify PCR products ranging from 400 to 500 base pairs. MTSP1 was subsequently found to be identical to matriptase (Genbank accession number AF118224; see also Takeuchi et al., *Proc. Natl. Acad. Sci. USA*, 96(20):11054-61 (1999); and Lin et al., *J. Biol. Chem.*, 274(26):18231-6 1999).

[0582] Using the protein sequence of the protease domain of the serine protease MTSP1, the EST database (dbEST) at the National Center for Biotechnology Information (Bethesda, Md.; www.ncbi.nlm.nih.gov) was searched for EST clones that contain similar or identical sequences to MTSP1 using the search algorithm tblastn. The tblastn algorithm compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands). The sequences for two identical EST clones (NCI\_CGAP\_Lu19 AI924527 and AI924182) derived from human lung tumor tissue showed 43% identity with the MTSP1 protein sequence. Subsequent search of GenBank and SwissProt database for the EST sequence AI924527 and AI924182 did not show any matching sequence to MTSP1, indicating that the sequence contained in these EST clones AI924527 and AI924182 may be portions of a new serine protease.

##### [0583] b. PCR Cloning of a cDNA Fragment of Another Membrane Type Serine Protease MTSP3

[0584] The double-stranded Marathon-Ready(tm) cDNA library derived from human lung carcinoma (LX-1) was obtained from Clontech (Palo Alto, Calif.; catalog # 7495-1) and used as a template. Two primers, 5'-TCACCGAGAA-GATGATGTGTGCAGGCATCC-3' (SEQ ID No:15) (sense primer), and 5'-GGGACAGGGGCTGTAAGGCAGG-GAATGAG-3' (SEQ ID No:16) (antisense primer), were used to amplify a ~360 bp DNA fragment. The PCR product was separated on a 2% agarose gel and purified using a gel extraction kit (catalog number 28706; QIAquick gel extraction kit; Qiagen). The purified DNA fragment was ligated into TA vectors (catalog number K4500-01; TOPO-TA cloning kit, Invitrogen, Carlsbad, Calif.). After transformation into *E. coli* cells, plasmids were isolated and analyzed by digestion with EcoRI restriction enzyme. Clones that had inserted DNA were further characterized by sequencing using a fluorescent dye-based DNA sequencing method (catalog number 4303149; BigDye terminator cycle sequencing kit with AmpliTaq DNA polymerase; Perkin Elmer, Lincoln, Calif.).

[0585] The DNA sequence obtained was analyzed and has 43% identity with the MTSP1 protein sequence. This indicates that the LX-1 cDNA library contains a desired nucleic acid molecule. It was used to isolate a cDNA clone encompassing a full length protease.

[0586] c. 5'- and 3'-Rapid Amplification of cDNA Ends (RACE)

[0587] To obtain the full-length cDNA that encoded this serine protease, hereafter called MTSP3, 5'- and 3'-RACE reactions were performed. The Marathon-Ready cDNA library from human lung carcinoma (LX-1) was used to isolate the 5' and 3' ends of the cDNA encoding MTSP3. Marathon-Ready cDNA is specifically made for RACE reactions. Two gene specific primers were used:

[0588] 5'-CCC GCAGCCATAGCCCCAGCTAACG-3' (SEQ ID No. 17) for 5'-RACE reaction and 5'-GCAGACGATGCGTACCAGGGGGAAGTC-3' (SEQ ID No. 18) for 3'-RACE reaction. Two fragments, approximately 1.8 kbp and 0.85 kbp, were isolated that correspond to the missing 5' and 3' end sequences, respectively. These fragments were subcloned as described above. They were further confirmed by Southern analysis using an internal cDNA fragment encompassing the 2 primers used in the RACE reactions as probe and by DNA sequence analysis.

[0589] d. PCR Amplification of cDNA Encoding Full-length Protease Domain of MTSP3

[0590] To obtain the cDNA fragment encoding the protease domain of MTSP3, an end-to-end PCR amplification using gene-specific primers was used. The two primers used were:

[0591] 5'-CTCGAGAAAAGAGTGGTGGGTGGGGAGGAGCCCTGTG-3' (SEQ ID No. 19) for the 5' end and

[0592] 5'-GCGGCCGCATTACAGCTCAGCCTTCCAGAC-3' (SEQ ID No. 20) for the 3' end. The 5' primer contains the sequence (underlined) that encodes the start of the MTSP3 protease domain (VVGGE<sup>E</sup>ASV). The 3' primer contains the stop codon (underlined) of MTSP3. A ~700-bp fragment was amplified and subcloned into a *Pichia pastoris* expression vector, pPIC9K.

[0593] e. C310S Mutagenesis of MTSP3

[0594] To eliminate the free cysteine (at position 310 in SEQ ID No. 4) that exists when the protease domain of the MTSP3 protein is expressed or the zymogen is activated, the free cysteine at position 310 (see SEQ ID No. 3), which is Cys122 if a chymotrypsin numbering scheme is used, was replaced with a serine. The resulting vector was designated pPIC9K:MTSP3C122S.

[0595] The gene encoding the protease domain of MTSP3 was mutagenized by PCR SOE (PCR-based splicing by overlap extension) to replace the unpaired cysteine at position 310 (122 chymotrypsin numbering system) with a serine. Two overlapping gene fragments, each containing the TCT codon for serine at position 310 were PCR amplified using the following primers: for the 5' gene fragment, TCTCTCGAGAAAAGAGTGTGGGGTGGGTGGGGAGGAGCCTCTGTG SEQ ID No. 51 and GCTCCTCATCAAAGAAGGGCAGAGAGATGGGCTGACTGTGCC SEQ ID No. 52; for the 3' gene fragment, ATTGCGGGCCGCATTACAGCTCAGCCTTCCAGAC (SEQ ID No. 53) and GGCACAGTCAGGCCCATCTCTGCCCCTTCTTTGATGAGGAGC (SEQ ID No. 54). The amplified gene fragments were purified on a 1% agarose gel, mixed and reamplified by PCR to produce the full length coding sequence for MTSP3 C122S. This sequence was then cut with restriction enzymes NotI and XhoI, and ligated into vector pPic9K.

[0596] 2. Sequence Analysis

[0597] All derived DNA and protein sequences were analyzed using MacVector (version 6.5; Oxford Molecular Ltd., Madison, Wis.). The full-length cDNA encoding MTSP3 is composed of 2,137 base pairs containing the longest open reading frame of 1,314 base pairs which translate to a 437-amino acid protein sequence. The cDNA fragment (nt 873-1,574) encoding the protease domain of MTSP3 is composed of 702 base pairs which translate to a 233-amino acid protein sequence plus the stop codon. The DNA sequence and the translated protein sequence of MTSP3 are shown in SEQ ID Nos. 3 and 4, respectively.

[0598] 3. Construction of the Expression Vectors

[0599] DNA encoding MTSP3 full length protein containing the C310S point mutation (i.e., MTSP3C122S) was cloned from pPIC9K:MTSP3C122S. The primers MTSP3:

[0600] 5'GAATTCATATGCCGCGCTTTAAAGTGTGGGTGGGGAGGAGGCC SEQ ID No. 47 (containing a NdeI restriction site) and MTSP3-3'GGGATACCCGTTACAGCTCAGCCTTCCAGAC 5' SEQ ID No. 48 (containing a BamHI restriction site) were used to PCR amplify the human MTSP3C122S protease domain utilizing a plasmin recognition sequence (PRFK) for zymogen activation. Amplification was conducted in a total volume of 50  $\mu$ l containing 10 mM KCl, 20 mM Tris-HCl (pH 8.8 at 25° C.), 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2.0 mM MgSO<sub>4</sub>, 0.1% Triton X-100, 0.3 mM dNTPs, 5.0 units of vent DNA polymerase, and 100 pmol of primers. The reaction mixtures were heated to 95° C. for 5 min, followed by 25-30 cycles of 95, 60, and 75° C. for 30 s each and a final extension at 75° C. for 2 min.

[0601] PCR products were purified using a QIAquick PCR purification kit (QIAGEN Inc., Chatsworth, Calif.). Full-length oligonucleotides were doubly digested with 10 units BamHI and 20 units NdeI for 2 h at 37° C. The digested fragments were purified on a 1.3% agarose gel and stained with ethidium bromide. The band containing the MTSP3C122S encoding DNA was excised and purified using a QIAEX II gel extraction kit.

[0602] The MTSP3C122S encoding DNA was then cloned into the NdeI and BamHI sites of the pET19b vector (Novagen) using standard methods. This vector allows the fusion of a HIS<sub>6</sub> tag for purification by metal affinity chromatography (MAC). Competent XL1 Blue cells (Stratagene) were transformed with the pET19b-MTSP3C122S vector and used to produce plasmid stocks. Proper insertion and DNA sequence were confirmed by fluorescent thermal dye DNA sequencing methods as well as restriction digests.

[0603] 4. Protein Expression, Purification, and Refolding

[0604] Overexpression of the gene product was achieved in *E. coli* strain BL21 (DE3) (Novagen, Madison Wis.) containing the DNAY plasmid for rare codon optimization (see, e.g., Garcia et al. (1986) *Cell* 45:453-459). Cells were grown at 37° C. in (2xYT) media supplemented with carbenicillin and kanamycin to a final concentrations of 50 ug/ml and 34 ug/ml, respectively. One liter cultures were inoculated with 10 mL of an overnight culture grown in the same media. Cells were allowed to grow to a density of 0.6-1.0 OD<sub>600</sub> before the addition of IPTG (final concentration 1.0 mM). Cells were grown an additional 4 hours before harvesting.

[0605] The cell pellet was resuspended in 20 mL of lysis buffer (50 mM Na<sub>2</sub>HPO<sub>4</sub>, 300 mM NaCl, pH 7.4). The cell

suspension was treated with 10-20 mg lysozyme and incubated at 37° C. for 1 hour. DNaseI was then added (1-2 mg) with mixing until the solution was no longer viscous. The solution was then transferred to a Rosette flask and sonicated, on ice, at high power for 15 min. Inclusion bodies were pelleted by centrifugation at 20K rpm (~48,000 g) at 4° C. for 30 min.

[0606] Inclusion bodies were washed by douncing 2 times in 50 mM Na<sub>2</sub>HPO<sub>4</sub>, 300 mM NaCl, 5% LADO, pH 7.4 followed by 2 times in 50 mM Na<sub>2</sub>HPO<sub>4</sub>, 300 mM NaCl, pH 7.4. Inclusion bodies (~500 mg) are solubilized in 25 mL 6 M GuHCl, 100 mM tris-HCl, 20 mM βMe, pH 8.0. This solution was spun at 20K rpm for 30 minutes to pull down any particulate matter. This solution was passed through a 0.2 μM filter and diluted to 100 mL in solubilization buffer.

[0607] MTSP3C122S was refolded by slowly adding the inclusion body mixture to 8 L of refolding buffer (100 mM tris-HCl, 150 mM NaCl, 5 mM GSH, 0.05 mM GSSG, 1 M arginine, pH 8.0) using a peristaltic pump. The refolding mixture was allowed to stir at 4° C. for 7 days or until the thiol concentration was below 1 mM as detected by Ellman's reagent. The solution was filtered through a 5 μM filter, concentrated by ultrafiltration and the buffer exchanged into MAC equilibration buffer (50 mM Na<sub>2</sub>HPO<sub>4</sub>, 300 mM NaCl, 10 mM imidazole, pH 8.0) by crossflow filtration. The resulting solution was passed through a 0.2 μM filter and further purified on a FPLC (Amersham-Pharmacia) using Pharmacia chelating sepharose. The solution was loaded onto the nickel loaded MAC at a flow rate of 1.0 mL/min and eluted with a linear gradient of 1.0 mM-1.0 M imidazole in 50 mM Na<sub>2</sub>HPO<sub>4</sub>, 300 mM NaCl, pH 8.0. Protein containing fractions were determined by SDS-PAGE and subsequently pooled and frozen at -80° C.

[0608] Small amounts of purified MTSP3C122S were activated using plasmin sepharose for 30 min. at 37° C. The resin was spun down at 14K rpm for 5 min. and the protein solution removed. The resulting solution was screened for activity against of series of protease substrates; spec-tpa, spec-pl, spec-UK, spec-fXIIa (American Diagnostica), S-2238, S-2266 (Kabi Diagnostica), S-2586, S-2366, S-2444, S-2288, S-2251, S-2302, S-2765, S-2222, spec-THE (Chromogenix), spec-fVIIa (Pentapharm). MTSP3C122S cleaved several of these substrates efficiently but was most active towards Spec-fXIIa, Spec-tPA, S-2765, Spec-fVIIa and S-2444.

[0609] 5. Gene Expression Profile of the Serine Protease MTSP3 in Normal and Tumor Tissues

[0610] To obtain information regarding the tissue distribution of the MTSP3 transcripts, the DNA insert encoding the MTSP3 protease domain was used to probe a RNA blot composed of 76 different human tissues (catalog number 7775-1; human multiple tissue expression (MTE) array; CLONTECH, Palo Alto, Calif.). The expression pattern observed in decreasing signal level was: trachea=colon (descending)=esophagus>colon (ascending)>colon (transverse)=rectum>ileum>duodenum>jejunum>bladder>ileocecum>stomach>kidney>appendix. It is also expressed less abundantly in fetal kidney, and in two tumor cell lines, HeLa S3 and leukemia, K-562. Northern analysis using RNA blots (catalog numbers 7780-1, 7765-1 & 7782-1; human 12-lane,

human muscle and human digestive system multiple tissue northern (MTN) blots; CLONTECH) confirmed that the expression was detected most abundantly in the colon, moderately in the esophagus, small intestine, bladder and kidney, and less abundantly in stomach and rectum. A single transcript of ~2.2 kb was detected.

[0611] Amplification of the MTSP3 transcript in several human primary tumors xenografted in mouse was performed using gene-specific primers. The MTSP3 transcript was detected in lung carcinoma (LX-1), colon adenocarcinoma (CX-1), colon adenocarcinoma (GI-112) and ovarian carcinoma (GI-102). No apparent signal was detected in another form of lung carcinoma (GI-1 17), breast carcinoma (GI-101), pancreatic adenocarcinoma (GI-103) and prostatic adenocarcinoma (PC3).

#### EXAMPLE 2

[0612] Identification of Genomic Clone of MTSP4

[0613] Using the nucleotide sequence encoding the protease domain of the serine protease MTSP1 (also called matriptase), the protein database (SWISSPROT) at the National Center for Biotechnology Information (Bethesda, Md.; <<http://www.ncbi.nlm.nih.gov>>) was searched for similar or identical sequence to MTSP1 using the search algorithm blastx. The blastx algorithm compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database. A protein encoding sequence (CAA18442) that has 37% identity to the MTSP1 protein sequence that was found to include a putative LDL-receptor domain and a trypsin-like serine protease domain was identified. This protein-encoding sequence (hereinafter referred to as MTSP4) was found to be encoded by a genomic clone (AL022314) derived from human chromosome 22 sequenced by the Sanger Centre Chromosome 22 Mapping Group and deposited into the public database as part of the Human Genome Project. Subsequent search of the GenBank database showed that no identical sequence has been deposited. A search of the EST database also did not show any matching human sequence, indicating that no human EST clone exists in the public database. Mouse EST clones (AI391417 and AA208793) are present and showed 88% identity to the serine protease at the nucleotide level.

[0614] PCR Cloning of a Genomic DNA Fragment of MTSP4 for Use as Hybridization Probe

[0615] In order to obtain tissue distribution profile of MTSP4 as well as to identify a tissue source for subsequent cloning of the cDNA, a genomic fragment was amplified from human genomic DNA using two gene-specific primers, 5'-CCTCCACGGTGTCTGTGGACCGTGTTC-3' (5' primer) SEQ ID No. 21 and 5'-CCTCGCGCAAGGCGC-CCCAGCCCCG-3' (3' primer) SEQ ID No. 22. These two primers amplified a 265-base pair fragment within a single exon of MTSP4. The fragment was then used as a hybridization probe on human tissue northern blot (human 12-lane multiple tissue northern (MTN) blot (catalog number 7780-1); CLONTECH, Palo Alto, Calif.). A prominent band (~2.6 kb) was detected in liver. Relatively weaker signals were obtained from the brain, heart, skeletal muscle and kidney. Since human liver showed a very strong signal, this tissue was selected for the amplification of the MTSP4 cDNA.

[0616] 5'- and 3'-Rapid Amplification of cDNA Ends (RACE)

[0617] To obtain a full-length clone encoding MTSP4, 5'- and 3'-RACE reactions were performed. The Marathon-Ready cDNA library from human liver (CLONTECH) was used to isolate the 5' and 3' ends of the cDNA encoding MTSP4. Marathon-Ready cDNA clones are specifically made for RACE reactions. Two gene specific primers were used: 5'-GCGTGGCGTCACCTGGTAGCGATAGAC-CTCGC-3' (SEQ ID No. 23) for 5'-RACE reaction and 5'-CCTCCACGGTGCTGTGGACCGTGTCC-3' (SEQ ID No. 24) for 3'-RACE reaction. No fragment was obtained from the initial 5'-RACE reaction.

[0618] The 3'-RACE reaction, however, produced a ~1.5 kbp fragment. A nested PCR reaction was used on the initial 5'-RACE reaction products to obtain part of the 5' end of MTSP4. The nested 5' gene-specific primer used was 5'-CCTCGCGCAAGGCGCCCCAGCCCCG-3' (SEQ ID No. 25) and produced a ~0.8 kbp fragment. The fragments were subcloned into pCR2.1-TOPO TA cloning vector (Invitrogen, Carlsbad, Calif.). The resulting clones were analyzed by Southern analysis using the internal genomic fragment encompassing the primers used in the RACE reactions as probe and by DNA sequence analysis. Sequence analysis of the 5'-RACE product showed that the potential initiation codon was still missing.

[0619] To obtain the 5' cDNA end that encodes the N terminus of MTSP4, the publicly available genomic sequence of chromosome 22 was searched for sequence corresponding to the sequence obtained in the 5'-RACE clone. The resulting genomic sequence was translated and the protein sequence was compared to that derived from the translated sequence of the 5'-RACE clone. After determining the overlapping sequences, a gene-specific oligonucleotide primer

[0620] (5'-TCAATCGGCCAGAGGGTGATCAGTGAG-3') SEQ ID No. 26

[0621] corresponding to the sequence upstream of the potential initiation codon and another gene-specific oligonucleotide primer

[0622] (5'-CCTCCTCAGTGCATAGGCATCAAAC-CAG-3') SEQ ID No. 27

[0623] corresponding to a sequence within the overlapping region were used to amplify the missing 5' cDNA of MTSP4 from the human liver cDNA library.

[0624] Splice Variants and Domain Organization of MTSP4

[0625] At least two cDNA fragments were consistently obtained during PCR amplification, indicating multiple splice variants of MTSP4. Subcloning and sequence analysis revealed that a longer, more abundant form, MTSP4-L and a shorter form, MTSP4-S. The encoded proteins are multi-domain, type II membrane-type serine proteases and include a transmembrane domain at the N terminus followed by a CUB domain, 3 LDLR domains and a trypsin-like serine protease domain at the C terminus. The difference between these two forms of MTSP4 is the absence in MTSP4-S of a 432-bp nucleotide sequence between the transmembrane and the CUB domains (see FIG. 2).

[0626] PCR Amplification of cDNA Encoding Full-length Protease Domain of MTSP4

[0627] To obtain a cDNA fragment encoding the protease domain of MTSP4, an end-to-end PCR amplification using gene-specific primers and the Marathon-Ready cDNA library from human liver was used. The two primers used were:

[0628] 5'-TCTCTCGAGAAAAGAATTGTTGGTG-GAGCTGTGTCCTCCGAG-3' (SEQ ID No. 28) for the 5' end and

[0629] 5'-AGGTGGGCCTTGCTTTG-CAGGGGGCAGTTC-3' for the 3' end SEQ ID NO. 29). The 5' primer contained the sequence that encodes the start of the MTSP4 protease domain (IVGGAVSSE). The 3' primer corresponds to the sequence just downstream of the stop codon. A ~740-bp fragment was amplified, subcloned into pCR2.1-TOPO TA cloning vector and sequenced.

[0630] Gene Expression Profile of MTSP4 in Normal and Tumor Tissues

[0631] To obtain information regarding the gene expression profile of the MTSP4 transcript, a DNA fragment encoding part of the LDL receptor domain and the protease domain was used to probe an RNA blot composed of 76 different human tissues (catalog number 7775-1; human multiple tissue expression (MTE) array; CLONTECH). As in the northern analysis of gel blot, a very strong signal was observed in the liver. Signals in other tissues were observed in (decreasing signal level): fetal liver>heart=kidney=adrenal gland=testis=fetal heart and kidney=skeletal muscle=bladder=placenta>brain=spinal cord=colon=stomach=spleen=lymph node=bone marrow=trachea=uterus=pancreas=salivary gland=mammary gland=lung. MTSP4 is also expressed less abundantly in several tumor cell lines including HeLa S3=leukemia K-562=Burkitt's lymphomas (Raji and Daudi)=colorectal adenocarcinoma (SW480)>lung carcinoma (A549)=leukemia MOLT-4=leukemia HL-60. PCR of the MTSP4 transcript from cDNA libraries made from several human primary tumors xenografted in nude mice (human tumor multiple tissue cDNA panel, catalog number K1522-1, CLONTECH) was performed using MTSP4-specific primers. The MTSP4 transcript was detected in breast carcinoma (GI-101), lung carcinoma (LX-1), colon adenocarcinoma (GI-12) and pancreatic adenocarcinoma (GI-103). No apparent signal was detected in another form of lung carcinoma (GI-117), colon adenocarcinoma (CX-1), ovarian carcinoma (GI-102). and prostatic adenocarcinoma (PC3). The MTSP4 transcript was also detected in LNCaP and PC-3 prostate cancer cell lines as well as in HT-1080 human fibrosarcoma cell line.

[0632] Sequence Analysis

[0633] MTSP4 DNA and protein sequences were analyzed using MacVector (version 6.5; Oxford Molecular Ltd., Madison, Wis.). The ORF of MTSP4-L includes 2,409 bp, which translate to a 802-amino acid protein, while the ORF of MTSP4-S is composed of 1,977 bp which translate to a 658-amino acid protein. The cDNA encoding the protease domain in both forms is composed of 708 bp which translate to a 235-amino acid protein sequence (see, SEQ ID No. 6) The DNA sequences and the translated protein sequences of MTSP4-L and MTSP4-S, and of the protease domain of MTSP4 are set forth in SEQ ID Nos. 8, 10 and 6, respectively.

## EXAMPLE 3

[0634] Cloning of MTSP6

[0635] Identification of Genomic Clone of MTSP6

[0636] Using the protein sequence of the protease domain of the serine protease MTSP4 (see EXAMPLE 2), the non-redundant database (all non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF) at the National Center for Biotechnology Information (Bethesda, Md.; <<http://www.ncbi.nlm.nih.gov>>) was searched for sequences that were similar or identical to MTSP4 using the search algorithm tblastn. The tblastn algorithm compares a protein query sequence against a nucleotide sequence database dynamically translated in all reading frames. A protein (55 amino acids), which has 60% identity with the query MTSP4 sequence (55 amino acids), was obtained from the translation of genomic sequence of AC015555 (nucleotide #15553 to 15717). This protein hereafter is referred to as MTSP6. Subsequent search of the GenBank database showed that no cDNA encoding MTSP6 has been deposited.

[0637] The gene exhibiting highest homology to MTSP6 was human transmembrane serine protease 2 (GenBank accession number U75329; Swissprot accession number O15393), which showed 66% identity to MTSP6 within the 45 amino acid regions compared. Consequently, the nucleotide sequence encoding the MTSP6 protease domain was obtained by comparing the protein sequence of human transmembrane serine protease 2 protease domain with the nucleotide sequence of AC015555 translated in six reading frames. The protein sequence obtained from the translated nucleotide sequence of MTSP6 revealed an overall 50% identity with human transmembrane serine protease 2. A search of the EST database indicated the presence of seven MTSP6 EST clones (AA883068, AW591433, AI978874, AI469095, AI935487, AA534591 and AI758271).

[0638] Cloning of Human MTSP6 Full-length cDNA

[0639] To obtain cDNA encoding the region of the MTSP6 protease domain identified by database searches described above, two gene-specific primers, Ch17-NSP-1, 5'-TCACG-CATCGTGGGTGGAACATGTCC-3' (5' primer) SEQ ID NO. 30 and Ch17-NSP-2AS, 5'-ACCCACCTCCATCTGCTCGTGGATCC-3' SEQ ID NO. 31 (3' primer), were used for PCR. These two primers amplified a 708-base pair fragment from human mammary gland carcinoma cDNA (Clontech Marathon-Ready cDNA, Cat. No. 7493-1).

[0640] To obtain the remaining, unknown cDNA of MTSP6, 5'- and 3'-RACE reactions were performed on the human mammary gland carcinoma. Marathon-Ready cDNA is specifically made for RACE reactions. The first RACE reactions were performed by PCR using Marathon cDNA adaptor primer 1 (AP1) with gene specific primers, Ch17-NSP-2AS, 5'-ACCCACCTCCATCTGCTCGTGGATCC-3' SEQ ID NO. 31 for 5'-RACE reaction and Ch17-NSP-1, 5'-TCACGCATCGTGGGTGGAACATGTCC-3' SEQ ID NO. 30 for 3'-RACE reaction. The PCR products were purified from agarose gel. A second nested PCR was then performed using Marathon cDNA adaptor primer 2 (AP2) with gene specific primer Ch17-NSP-3AS, 5'-CCACAGC-CTCCTCTCTTGACACACCAG-3' SEQ ID No. 32 for 5'-RACE reaction (using first 5'-RACE product as template) and Ch17-NSP-3 5'-ACGCCCCTGTGGATCATCACT-GCTGC-3' SEQ ID No. 33 for 3'-RACE reaction (using first

3'-RACE product as template). First 5'- and 3'-RACE products were also used as template for PCR reactions using primers Ch17-NSP-3 and Ch17-NSP-4AS to obtain a cDNA fragment for use as a probe. PCR products from RACE reactions which were larger than 700 bp were cut out and purified from agarose gel and subcloned into pCR2.1-TOPO cloning vector (Invitrogen, Carlsbad, Calif.). Colony hybridization was then performed to identify positive colonies containing MTSP6 sequence. Positive clones were identified by colony hybridization using the 495 bp DNA fragment obtained from PCR reaction (with primers Ch17-NSP-3 and Ch17-NSP-4AS) and by DNA sequencing.

[0641] Sequence analysis of the 5'-RACE products indicated that an additional 420 bp of upstream sequence were obtained. The potential initial codon was not present in the 5'-RACE sequence. Another round of nested 5'-RACE reaction was performed using AP2 and a gene specific primer (designed based on the new RACE sequence) Ch17-NSP-5AS 5'-TCCCTCCCTCACATATACTGAGTGGTG-3' SEQ ID No. 34, using the PCR products obtained from the first 5'-RACE as template. A PCR product of 367 bp using Ch17-NSP-6 5'-CGACTGCTCAGGGAAGTCAGAT-GTCG-3' SEQ ID NO. 35 (designed based on the new 5'-RACE sequence) and Ch17-NSP-5AS was used to identify the positive clones. An additional sequence of 480 bp was obtained from the second 5'-RACE products. A potential ATG start codon was observed within a sequence of GTCACCATGG (nucleotides 262-272 of SEQ ID No. 12, which appears to be a Kozak sequence (GCC (A/G) CCAUGG), indicating that this ATG is likely the initiation codon for MTSP6.

[0642] The 3'-RACE reaction to obtain the rest of the 3' end of MTSP6 was not successful using Marathon Ready human mammary gland carcinoma cDNA. The sequence of the 3'-RACE products obtained was exclusively that of an MTSP6 cDNA truncated with the Marathon AP2 primer sequence within the coding region.

[0643] The 3'-end sequence of MTSP6 was obtained by PCR using Ch17-NSP-3 (5'-ACGCCCCTGTGGATCATCACTGCTGC-3'; SEQ ID NO. 33) and Ch17-NSP-4 (5'-CTGGTGTGTCAGAGAGAGGCTGTGG-3'; SEQ ID NO. 37) with an antisense primer Ch17-NSP-7AS (5'-ACT-CAGTGGTACTTATCCCCCTCCTC-3'; SEQ ID NO. 38) designed based on the sequence of an EST clone AA883068, which apparently covers the 3'-end of MTSP6 sequence, and human small intestine cDNA (Clontech) as template. Two PCR products (650 bp and 182 bp, respectively) were obtained and DNA sequence analysis indicated that both PCR products contained a stop codon.

[0644] Sequence Analysis and Domain Organization of MTSP6

[0645] The MTSP6 DNA and protein sequences were analyzed using DNA Strider (version 1.2). The ORF of MTSP6 is composed of 1,362 bp, which translate into a 453-amino acid protein. Protein sequence analysis using the SMART (Simple Modular Architecture Research Tool) program at <http://smart.embl-heidelberg.de> predicts that MTSP6 is a multi-domain, type-II membrane-type serine protease containing of a transmembrane domain (amino acids 48-68) at the N terminus followed by a LDLRa domain (LDL receptor domain class a) (amino acids 72-108), a SR

domain (Scavenger receptor Cys-rich domain)(amino acids 109-205), and a trypsin-like serine protease domain (amino acids 216-443) (see FIG. 3).

[0646] Gene Expression Profile of MTSP6 in Normal and Tumor Tissues

[0647] To obtain information regarding the gene expression profile of the MTSP6 transcript, a 495 bp DNA fragment obtained from PCR reaction with primers Ch17-NSP-3 and NSP-4AS was used to probe an RNA blot composed of 76 different human tissues (catalog number 7775-1; human multiple tissue expression (MTE) array; CLONTECH). The strongest signal was observed in duodenum. Signal in other tissues were observed in (decreased signal level): Stomach>trachea=mammary gland=thyroid gland=salivary gland=pituitary gland pancreas>kidney>lung>jejunum=ileum=ilocecum=appendix=fetal kidney>fetal lung. Very weak signals can also be detected in several other tissues. MTSP6 is also expressed in several tumor cell lines including HeLa S3>colorectal adenocarcinoma (SW480)>leukemia MOLT-4>leukemia K-562. PCR analysis of the MTSP6 transcript from cDNA libraries made from several human primary tumors xenografted in nude mice (human tumor multiple tissue cDNA panel, catalog number K1522-1, CLONTECH) was performed using MTSP6-specific primers (Ch17-NSP-3 and Ch17-NSP2AS). The MTSP6 transcript was strongly detected in lung carcinoma (LX-1), moderately detected in pancreatic adenocarcinoma (GI-103), weakly detected in ovarian carcinoma (GI-102); and very weakly detected in colon adenocarcinoma (GI-112 and CX-1), breast carcinoma (GI-101), lung carcinoma (GI-117) and prostatic adenocarcinoma (PC3). The MTSP6 transcript was also detected in breast cancer cell line MDA-MB-231, prostate cancer cell line PC-3, but not in HT-1080 human fibrosarcoma cell line. MTSP6 is also expressed in mammary gland carcinoma cDNA (Clontech).

#### EXAMPLE 4

[0648] Expression of the Protease MTSP Domains

[0649] The DNA encoding each of the MTSP 3 and 4 protease domains was cloned into a derivative of the *Pichia pastoris* vector pPIC9K (available from Invitrogen; see SEQ ID NO. 45). Plasmid pPIC9k features include the 5' AOX1 promoter fragment at 1-948; 5' AOX1 primer site at 855-875; alpha-factor secretion signal(s) at 949-1218; alpha-factor primer site at 1152-1172; multiple cloning site at 1192-1241; 3' AOX1 primer site at 1327-1347; 3' AOX1 transcription termination region at 1253-1586; HIS4 ORF at 4514-1980; kanamycin resistance gene at 5743-4928; 3' AOX1 fragment at 6122-6879; Cole1 origin at 7961-7288; and the ampicillin resistance gene at 8966-8106. The plasmid used herein is derived from pPIC9K by eliminating the XhoI site in the kanamycin resistance gene and the resulting vector is herein designated pPIC9KX.

[0650] Primers Used for PCR Amplification of Protease Domain and Subcloning into the XhoI/NotI Sites of *Pichia* Vector

[0651] MTSP3

[0652] 5' primer (with XhoI site [underlined]) SEQ ID No. 39

[0653] 5' TCTCTCGAGAAAAGAGTG-GTGGGTGGGGAGGAGGCCTCTGTG 3'

[0654] 3' primer (with NotI site [underlined]) SEQ ID No. 40

[0655] 5' ATTCGCGGCCGCATTACAGCTCAGCCT-TCCAGAC 3'

[0656] MTSP4-S and MTSP4-L

[0657] 5' primer (with XhoI site [underlined]) SEQ ID No. 41

[0658] 5' TCTCTCGAGAAAAGAATTGTTGGTG-GAGCTGTGTCCTCCGAG

[0659] 3' primer with NotI site SEQ ID No. 42

[0660] 5' ATTCGCGGCCGCTCAGGTCACCACT-TGCTGGATCCAG 3'

[0661] MTSP6

[0662] MTSP6 was cloned into the *E. coli* TOPO vector (pcR@ 2.1 TOPO™, SEQ ID No. 46, Invitrogen, Carlsbad, Calif.; the TOPO@ TA Cloning@ Kit is designed for cloning Taq-amplified PCR products).

[0663] 5' primer (with XhoI site [underlined]) SEQ ID No. 43

[0664] 5'CTCGAGAAACGCATCGTGGGTG-GAAACATGTCCTTG 3'

[0665] 3' primer NotI site comes from *E. coli* TOPO vector SEQ ID No. 44:

[0666] 5' ACTCAGGTGGCTACTTATCCCCTC-CTC 3'

#### EXAMPLE 5

[0667] Assays for Identification of Candidate Compounds that Modulate that Activity of an MTSP

[0668] Assay for Identifying Inhibitors

[0669] The ability of test compounds to act as inhibitors of catalytic activity of an MTSP, including MTSP1, MTSP3, MTSP4, MTSP6 can be assessed in an amidolytic assay. The inhibitor-induced inhibition of amidolytic activity by a recombinant MTSP or the protease domain portions thereof, can be measured by IC50 values in such an assay.

[0670] An exemplary assay buffer is HBSA (10 mM Hepes, 150 mM sodium chloride, pH 7.4, 0.1% bovine serum albumin). All reagents were from Sigma Chemical Co. (St. Louis, Mo.), unless otherwise indicated. Two IC50 assays at 30-minute (a 30-minute preincubation of test compound and enzyme) and at 0-minutes (no preincubation of test compound and enzyme) are conducted. For the IC50 assay at 30-minute, the following reagents are combined in appropriate wells of a Corning microtiter plate: 50 microliters of HBSA, 50 microliters of the test compound, diluted (covering a broad concentration range) in HBSA (or HBSA alone for uninhibited velocity measurement), and 50 microliters of the MTSP or protease domain thereof diluted in buffer, yielding a final enzyme concentration of about 100-500 pM. Following a 30-minute incubation at ambient temperature, the assay is initiated by the addition of 50 microliters of a substrate for the particular MTSP (see, e.g., table and discussion below) and reconstituted in deionized water, followed by dilution in HBSA prior to the assay) were

added to the wells, yielding a final volume of 200 microliters and a final substrate concentration of 300  $\mu$ M (about 1.5-times Km).

[0671] For an IC50 assay at 0-minute, the same reagents are combined: 50 microliters of HBSA, 50 microliters of the test compound, diluted (covering the identical concentration range) in HBSA (or HBSA alone for uninhibited velocity measurement), and 50 microliters of the substrate, such as a chromogenic substrate. The assay is initiated by the addition of 50 microliters of MTSP. The final concentrations of all components are identical in both IC50 assays (at 30- and 0-minute incubations).

[0672] The initial velocity of the substrate hydrolysis is measured in both assays by, for example for a chromogenic substrate, as the change of absorbance at a particular wavelength, using a Thermo Max $\Delta$ Kinetic Microplate Reader (Molecular Devices) over a 5 minute period, in which less than 5% of the added substrate was used. The concentration of added inhibitor, which caused a 50% decrease in the initial rate of hydrolysis was defined as the respective IC50 value in each of the two assays (30- and 0-minute).

[0673] Another Assay for Identifying Inhibitors

[0674] Test compounds for inhibition of the protease activity of the protease domain of is assayed in Costar 96 well tissue culture plates (Corning N.Y.). Approximately 2-3 nM the MTSP or protease domain thereof is mixed with varying concentrations of inhibitor in 29.2 mM Tris, pH 8.4, 29.2 mM imidazole, 217 mM NaCl (100 mL final volume), and allowed to incubate at room temperature for 30 minutes. 400 mM substrate is added, and the reaction monitored in a SpectraMAX Plus microplate reader (Molecular Devices, Sunnyvale Calif.) by following the change in a parameter correlated with hydrolysis, such as absorbance for a chromogenic substrate for 1 hour at 37° C.

[0675] Assay for Screening MTSP6

[0676] The protease domain of MTSP6 expressed in *Pichia pastoris* is assayed for inhibition by various compounds in Costar 96 well tissue culture plates (Corning N.Y.). Approximately 1-20 nM MTSP6 is mixed with varying concentrations of inhibitor in 29.2 mM Tris, pH 8.4, 29.2 mM Imidazole, 217 mM NaCl (100  $\mu$ L final volume), and allowed to incubate at room temperature for 30 minutes. 500  $\mu$ M substrate Spectrozyme t-PA (American Diagnostica, Greenwich, Conn.) is added, and the reaction is monitored in a SpectraMAX Plus microplate reader (Molecular Devices, Sunnyvale Calif.) by measuring the change in absorbance at 405 nm for 30 minutes at 37° C.

[0677] Identification of Substrates

[0678] Particular substrates for use in the assays can be identified empirically by testing substrates. The following list of substrates are exemplary of those that can be tested.

Substrate name	Structure
S 2366	pyroGlu-Pro-Arg-pNA.HCl
spectrozyme t-PA	CH <sub>3</sub> SO <sub>2</sub> -D-HHT-Gly-Arg-pNA.AcOH
N-p-tosyl-Gly-Pro-Arg-pNA	N-p-tosyl-Gly-Pro-Arg-pNA

-continued

Substrate name	Structure
Benzoyl-Val-Gly-Arg-pNA	Benzoyl-Val-Gly-Arg-pNA
Pefachrome t-PA	CH <sub>3</sub> SO <sub>2</sub> -D-HHT-Gly-Arg-pNA
S 2765	N- $\alpha$ -Z-D-Arg-Gly-Arg-pNA.2HCl
S 2444	pyroGlu-Gly-Arg-pNA.HCl
S 2288	H-D-Ile-Pro-Arg-pNA.2HCl
spectrozyme UK	Cbo-L-( $\gamma$ )Glu( $\alpha$ -t-BuO)-Gly-Arg-pNA.2AcOH
S 2302	H-D-Pro-Phe-Arg-pNA.2HCl
S 2266	H-D-Val-Leu-Arg-pNA.2HCl
S 2222	Bz-Ile-Glu(g-OR)-Gly-Arg-pNA.HCl R = H(50%) and R = CH <sub>3</sub> (50%)
Chromozyme PK	Benzoyl-Pro-Phe-Arg-pNA
S 2238	H-D-Phe-Pip-Arg-pNA.2HCl
S 2251	H-D-Val-Leu-Lys-pNA.2HCl
Spectrozyme PI	H-D-Nle-HHT-Lys-pNA.2AcOH Pyr-Arg-Thr-Lys-Arg-AMC H-Arg-Gln-Arg-Arg-AMC Boc-Gln-Gly-Arg-AMC Z-Arg-Arg-AMC
Spectrozyme THE	H-D-HHT-Ala-Arg-pNA.2AcOH
Spectrozyme EXIIa	H-D-CHT-Gly-Arg-pNA.2AcOH CVS 2081-6 (MeSO <sub>2</sub> -dPhe-Pro-Arg-pNA) Pefachrome fVIIa (CH <sub>3</sub> SO <sub>2</sub> -D-CHA-But-Arg-pNA)

pNA = para-nitranilide (chromogenic)

AMC = amino methyl coumarin (fluorescent)

[0679] If none of the above substrates are cleaved, a coupled assay, described above, can be used. Briefly, test the ability of the protease to activate and enzyme, such as plasminogen and trypsinogen. To perform these assays, the single chain protease is incubated with a zymogen, such as plasminogen or trypsinogen, in the presence of the a known substrate, such, lys-plasminogen, for the zymogen. If the single chain activates the zymogen, the activated enzyme, such as plasmin and trypsin, will degrade the substrate therefor.

#### EXAMPLE 6

[0680] Isolation and Cloning of Matriptase

[0681] A. Cell Type and Growth of Cells

[0682] Human prostate adenocarcinoma cell line, PC-3, was purchased from ATCC (catalog number CRL-1435; Manassas, Va.). The cells were cultured at 37° C., 5% CO<sub>2</sub> in Ham's F-12K growth medium (catalog number 9077; Irvine) supplemented with 2 mM L-glutamine and 10% fetal bovine serum. All subsequent cell manipulations were carried out according to the manufacturer's instructions. PC-3 cells were allowed to grow to about 90% confluence, and were then washed briefly with 1xphosphate buffered saline.

[0683] B. Isolation of Total RNA, and Purification and Enrichment of polyA<sup>+</sup> RNA

[0684] PC-3 cells were lysed in Trizol reagent (catalog number 15596; Life Technologies, Rockville, Md.) and total RNA was isolated according to the manufacturer's protocol. The concentration of total RNA was estimated from absorbance reading at 260 nm. PolyA<sup>+</sup> RNA was purified and enriched using oligo-dT beads (catalog number 70061; Oligotex, Qiagen, Valencia, Calif.).

**[0685]** C. Reverse-transcription and Polymerase Chain Reaction (PCR)

**[0686]** PC-3-derived polyA<sup>+</sup> RNA was converted to single-stranded cDNA (sscDNA) by reverse transcription using ProSTAR first-strand RT-PCR kit (catalog number 200420; Stratagene, La Jolla, Calif.) and SuperScript II RNase H<sup>-</sup> reverse transcriptase (catalog number 18064-022; Life Technologies). After reverse transcription, an aliquot of PC-3 sscDNA (4  $\mu$ L) was subjected to PCR using 2 mM each of the sense and anti-sense degenerate oligonucleotide primers and Taq polymerase (catalog number 201203; Qiagen). Total reaction volume was 100  $\mu$ L. The sequence of the sense primer was 5'TGGRT(I)VT(I)WS(I)GC(I)R-C(I)CAYTG-3' (SEQ ID No. 13) and that of the anti-sense was 5'-(I)GG(I)CC(I)CC(I)SWRTC(I)CCYT(I)RCA(I)GH-RTC-3' (SEQ ID No. 14), where R=A,G; V=G,A,C; W=A,T; S=G,C; Y=C,T; H=A,T,C. The primer sequences correspond to two highly conserved regions in all chymotrypsin-like serine proteases and amplify PCR products ranging from approximately 400 to 500 base pairs.

**[0687]** D. Clone Screening and Sequencing

**[0688]** The PCR products were separated on a 2% agarose gel and purified using a gel extraction kit (catalog number 28706; QIAquick gel extraction kit; Qiagen). The purified DNA fragments were ligated into pCR2.1-TOPO (catalog number K4500-01; Invitrogen, Carlsbad, Calif.). After transformation into *E. Coli* cells, plasmid DNA was isolated and analyzed by digestion with EcoRI restriction enzyme. Clones that had inserted nucleic acid were further characterized by sequencing using a fluorescent dye-based DNA sequencing method (catalog number 4303149; BigDye terminator cycle sequencing kit with AmpliTaq DNA polymerase; Perkin Elmer, Lincoln, Calif.). A total of 31 clones were sequenced and analyzed. All sequences were analyzed by a multiple nucleotide sequence alignment algorithm (blastn) ([www.ncbi.nlm.nih.gov/blast](http://www.ncbi.nlm.nih.gov/blast)) to identify identical or closely related DNA deposited in GenBank (NCBI, Bethesda, Md.). Those that did not show significant homology were further analyzed using blastx, which compares the six-frame conceptual translation products of a nucleotide sequence (both strands) against a protein sequence database (SwissProt). Eight clones yielded identical cDNA fragments that encode MTSP1. MTSP1 was subsequently found to be identical to matriptase (GenBank accession number AF118224).

**[0689]** E. Rapid Amplification of cDNA Ends (RACE) and Gene-specific Amplification of MTSP1

**[0690]** To obtain DNA encoding the complete protease domain of MTSP1, RACE and gene-specific amplification reactions were performed. A human prostate Marathon-Ready cDNA (catalog # 7418-1; Clontech) was used to isolate part of the cDNA encoding MTSP1. Marathon-Ready cDNA is prepared to contain a known hybridization sequence at the 5' and 3' ends of the sscDNA. The 3' region of MTSP1 cDNA was obtained by a 3'-RACE reaction using a gene specific primer, 5'-CACCCCTTCTCAATGACTTCACCTTCG-3' (SEQ ID No. 55). The 5' end of the MTSP1 protease domain was obtained by gene-specific amplification reaction using two MTSP1-specific primers, 5'-TACCTCTCTACGACTCC-3' (SEQ ID No. 56) for the sense primer and 5'-GAGGTTCTCGCAGGTGGTCTG-GTTG-3' (SEQ ID No. 57) for the antisense primer. The sequences for these two primers were obtained from the human SNC19 mRNA sequence. The 3'-RACE reaction and gene-specific PCR produced DNA fragments that were >1

kbp in size. These fragments were subcloned into pCR2.1-TOPO (Invitrogen, San Diego, Calif.). After transformation into *E. coli* cells, plasmid DNA was isolated and analyzed by digestion with EcoRI restriction enzyme. Clones that had inserts were characterized by Southern blot analysis (using the internal cDNA fragment as probe) and by DNA sequence analysis.

**[0691]** F. PCR Amplification of cDNA Encoding the Protease Domain of MTSP1

**[0692]** To obtain a cDNA fragment encoding the entire protease domain of MTSP1, an end-to-end PCR amplification using gene-specific primers was used. The two primers used were: 5'-CTCGAGAAAAGAGTTGT-TGGGGGCACGGATGCGGATGAG-3' (SEQ ID No. 58) for the 5' end and 5'-GCGGCCGCACTATACCCCAGTGT-TCTCTTTGATCCA-3' (SEQ ID No. 36 for the 3' end. The 5' primer contained the sequence that encodes the start of the MTSP1 protease domain (VVGTDAD) (SEQ. ID. NO. 10). The 3' primer contained the stop codon of MTSP1. A ~800-bp fragment was amplified, purified and subcloned into the *Pichia pastoris* expression vector, pPIC9K, resulting in pPIC9K-MTSP1.

**[0693]** G. Gene Expression Profile of MTSP1 in Normal Tissues, Cancer Cells and Cancer Tissues

**[0694]** To obtain information regarding the tissue distribution and gene expression level of MTSP1, the DNA insert from pPIC9K-MTSP1 was used to probe a blot containing RNA from 76 different human tissues (catalog number 7775-1; human multiple tissue expression (MTE) array; CLONTECH, Palo Alto, Calif.). Significant expression was observed in the colon (ascending, transverse and descending), rectum, trachea, esophagus and duodenum. Moderate expression levels were observed in the jejunum, ileum, ilocecum, stomach, prostate, pituitary gland, appendix, kidney, lung, placenta, pancreas, thyroid gland, salivary gland, mammary gland, fetal kidney, and fetal lung. Lower expression levels were seen in the spleen, thymus, peripheral blood leukocyte, lymph node, bone marrow, bladder, uterus, liver, adrenal gland, fetal heart, fetal liver, fetal spleen, and fetal thymus. A significant amount of the MTSP1 transcript was also detected in colorectal adenocarcinoma cell line (SW480), Burkitt's lymphoma cell line (Daudi), and leukemia cell line (HL-60). RT-PCR of the MTSP1 transcript in several human primary tumors xenografted in athymic nude mice was performed using gene-specific primers. A high level of MTSP1 transcript was detected in colon adenocarcinoma (CX-1) and pancreatic adenocarcinoma (GI-103). Moderate levels were observed in another colon adenocarcinoma (GI-112), ovarian carcinoma (GI-102), lung carcinoma (LX-1), and breast carcinoma (GI-101). Another lung carcinoma (GI-117) expressed a low level of the MTSP1 transcript. A similar RT-PCR was performed to detect the presence of the MTSP1 transcript in PC-3 and LNCaP cell lines. Both cell lines expressed significant amounts of MTSP1 transcript.

**[0695]** H. Sequence Analysis

**[0696]** All derived DNA and protein sequences were analyzed using MacVector (version 6.5; Oxford Molecular Ltd., Madison, Wis.). The cDNA encoding the protease domain of MTSP1 is composed of 726 base pairs which translate into a 241-amino acid protein sequence (rMAP) (see SEQ ID No. 1, 2, 49 and 50).

## EXAMPLE 7

[0697] Production of Recombinant Serine Protease Domain of Matriptase or MTSP1 (rMAP)

[0698] A. Fermentation

[0699] The production of multi-milligram amounts of rMAP was carried out by fermentation in a BioFlo 3000 fermentor (New Brunswick Scientific, NJ) equipped with a 3.3 L capacity bioreactor using a SMD1168/pPIC9K:MTSP1 Sac SC1 clone. ZA001 complex media (10 g/L yeast extract, 20 g/L peptone, 40 g/L glycerol, 5 g/L ammonium sulfate, 0.2 g/L calcium sulfate dihydrate, 2 g/L magnesium sulfate heptahydrate, 2 g/L potassium sulfate, 25 g/L sodium hexametaphosphate, 4.35 ml/L PTM1) was inoculated with 100 ml of an overnight culture of the *P. pastoris* transformant. The culture was supplemented with 50% glycerol by fed-batch phase and induced for 18-24 hours with methanol controlled at 0.025%.

[0700] B. Purification of Recombinant Serine Protease Domain of Matriptase or MTSP1 (rMAP)

[0701] The rMAP was secreted into the culture medium, so the first step of the purification involved the removal of cells and cell debris by centrifugation at 5000 g for 30 minutes. The resulting supernatant was decanted, adjusted to pH 8.0 with 10 N NaOH, and filtered through a SartoBran 300 0.45+0.2  $\mu$ M capsule. This supernatant was concentrated to 1 L by ultrafiltration using a 10 kDa ultrafiltration cartridge (NC SRT UF system with AG/Technologies UFP-10-C-5A filter), and the buffer was exchanged by crossflow filtration into 50 mM tris-HCl, 50 mM NaCl, 0.05% tween-80, pH 8.0 (buffer A). The filtration unit was rinsed once with 1 L buffer A which was combined with the concentrate.

[0702] The concentrated rMAP-containing solution was passed over a 150 ml benzamidine column that had been equilibrated with buffer A, at a flow rate of 8 ml/min. The column was washed with 3 column volumes of 50 mM tris-HCl, 1.0 M NaCl, 0.05% tween-80, pH 8.0 (buffer B) and eluted with 3 column volumes of 50 mM tris-HCl, 1.0 M L-arginine, 0.05% tween-80, pH 8.0 (buffer C). Fractions containing rMAP were identified by activity assay and pooled. This pooled material was concentrated to 10 ml using a JumboSep concentrator (Pall Gelman) and a 10 kDa cutoff membrane. Once concentrated to 10 ml, the buffer was exchanged into 50 mM Na<sub>2</sub>HPO<sub>4</sub>, 125 mM NaCl, pH 5.5 (buffer D) and the volume adjusted to 5-10 ml. The retentate was removed and the concentrator washed with buffer D which was added to the concentrate. The total sample volume was adjusted 15 ml.

[0703] The partially purified rMAP was passed through a 5 ml Q-sepharose Fast Flow HiTrap column (Amersham-Pharmacia Biotech) pre-equilibrated with 15 ml of buffer D. The flow through was collected. The HiTrap column was washed with an additional 10 ml of buffer D. Both flow throughs were pooled, and the protein concentration was determined by measurement of OD<sub>280</sub> (using an extinction coefficient of 2.012 mg/OD<sub>280</sub>). Purified rMAP was then deglycosylated by the addition 0.1  $\mu$ l of Endoglycosidase H (ProZyme, 5 U/ml) per mg of protein and incubating overnight at 4° C. with gentle swirling.

[0704] The conductivity of the deglycosylated pool was adjusted to 2.0-3.0 mS/cm with Nanopure H<sub>2</sub>O and the pH

adjusted to 6.5 (-200-300 mL final volume). The rMAP was then further purified by anion exchange chromatography by loading directly onto a Pharmacia Akta Explorer system using a 7 mL Source 15Q anion exchange column (Amersham-Pharmacia Biotech). The protein was eluted in a buffer containing 50 mM HEPES, pH 6.5 with a 0-0.33 M NaCl gradient over 10 column volumes at a flow rate of 6 ml/min. Fractions containing protein were pooled, and benzamidine was added to a final concentration of 10 mM. Protein purity was examined by SDS-PAGE and protein concentration determined by measurement of OD<sub>280</sub> and use of a theoretical extinction coefficient of 2.012 mg/OD<sub>280</sub>.

## EXAMPLE 8

[0705] Assays

[0706] Amidolytic Assay for Determining Inhibition of Serine Protease Activity of Matriptase or MTSP1

[0707] The ability of test compounds to act as inhibitors of rMAP catalytic activity was assessed by determining the inhibitor-induced inhibition of amidolytic activity by the MAP, as measured by IC<sub>50</sub> values. The assay buffer was HBSA (10 mM Hepes, 150 mM sodium chloride, pH 7.4, 0.1% bovine serum albumin). All reagents were from Sigma Chemical Co. (St. Louis, Mo.), unless otherwise indicated.

[0708] Two IC<sub>50</sub> assays (a) one at either 30-minutes or 60-minutes (a 30-minute or a 60-minute preincubation of test compound and enzyme) and (b) one at 0-minutes (no preincubation of test compound and enzyme) were conducted. For the IC<sub>50</sub> assay at either 30-minutes or 60-minutes, the following reagents were combined in appropriate wells of a Corning microtiter plate: 50 microliters of HBSA, 50 microliters of the test compound, diluted (covering a broad concentration range) in HBSA (or HBSA alone for uninhibited velocity measurement), and 50 microliters of the rMAP (Corvas International) diluted in buffer, yielding a final enzyme concentration of 250 pM as determined by active site filtration. Following either a 30-minute or a 60-minute incubation at ambient temperature, the assay was initiated by the addition of 50 microliters of the substrate S-2765 (N- $\alpha$ -Benzyloxycarbonyl-D-arginyl-L-glycyl-L-arginine-p-nitroaniline dihydrochloride; DiaPharma Group, Inc.; Franklin, Ohio) to each well, yielding a final assay volume of 200 microliters and a final substrate concentration of 100  $\mu$ M (about 4-times K<sub>m</sub>). Before addition to the assay mixture, S-2765 was reconstituted in deionized water and diluted in HBSA. For the IC<sub>50</sub> assay at 0 minutes; the same reagents were combined: 50 microliters of HBSA, 50 microliters of the test compound, diluted (covering the identical concentration range) in HBSA (or HBSA alone for uninhibited velocity measurement), and 50 microliters of the substrate S-2765. The assay was initiated by the addition of 50 microliters of rMAP. The final concentrations of all components were identical in both IC<sub>50</sub> assays (at 30- or 60- and 0-minute).

[0709] The initial velocity of chromogenic substrate hydrolysis was measured in both assays by the change of absorbance at 405 nM using a Thermo Max® Kinetic Microplate Reader (Molecular Devices) over a 5 minute period, in which less than 5% of the added substrate was used. The concentration of added inhibitor, which caused a 50% decrease in the initial rate of hydrolysis was defined as the respective IC<sub>50</sub> value in each of the two assays (30- or 60-minutes and 0-minute).

[0710] In Vitro Enzyme Assays for Specificity Determination

[0711] The ability of compounds to act as a selective inhibitor of matriptase activity was assessed by determining the concentration of test compound that inhibits the activity of matriptase by 50%, ( $IC_{50}$ ) as described in the above Example, and comparing  $IC_{50}$  value for matriptase to that determined for all or some of the following serine proteases: thrombin, recombinant tissue plasminogen activator (rt-PA), plasmin, activated protein C, chymotrypsin, factor Xa and trypsin.

[0712] The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin). The assay for  $IC_{50}$  determinations was conducted by combining in appropriate wells of a Corning microtiter plate, 50 microliters of HBSA, 50 microliters of the test compound at a specified concentration (covering a broad concentration range) diluted in HBSA (or HBSA alone for  $V_0$  (uninhibited velocity) measurement), and 50 microliters of the enzyme diluted in HBSA. Following a 30 minute incubation at ambient temperature, 50 microliters of the substrate at the concentrations specified below were added to the wells, yielding a final total volume of 200 microliters. The initial velocity of chromogenic substrate hydrolysis was measured by the change in absorbance at 405 nm using a Thermo Max® Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was used. The concentration of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the  $IC_{50}$  value.

[0713] Thrombin (fIIa) Assay

[0714] Enzyme activity was determined using the chromogenic substrate, Pefachrome t-PA ( $CH_3SO_2$ -D-hexahydrotyrosine-glycyl-L-Arginine-p-nitroaniline, obtained from Pentapharm Ltd.). The substrate was reconstituted in deionized water prior to use. Purified human  $\alpha$ -thrombin was obtained from Enzyme Research Laboratories, Inc. The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

[0715]  $IC_{50}$  determinations were conducted where HBSA (50  $\mu$ L),  $\alpha$ -thrombin (50  $\mu$ L) (the final enzyme concentration is 0.5 nM) and inhibitor (50  $\mu$ L) (covering a broad concentration range), were combined in appropriate wells and incubated for 30 minutes at room temperature prior to the addition of substrate Pefachrome-t-PA (50  $\mu$ L) (the final substrate concentration is 250  $\mu$ M, about 5 times  $K_m$ ). The initial velocity of Pefachrome t-PA hydrolysis was measured by the change in absorbance at 405 nm using a Thermo Max® Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was used. The concentration of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the  $IC_{50}$  value.

[0716] Factor Xa

[0717] Factor Xa catalytic activity was determined using the chromogenic substrate S-2765 (N-benzoyloxycarbonyl-D-arginine-L-glycine-L-arginine-p-nitroaniline), obtained from DiaPharma Group (Franklin, Ohio). All substrates were reconstituted in deionized water prior to use. The final concentration of S-2765 was 250  $\mu$ M (about 5-times  $K_m$ ). Purified human Factor X was obtained from Enzyme

Research Laboratories, Inc. (South Bend, Ind.) and Factor Xa (FXa) was activated and prepared from it as described [Bock, P. E., Craig, P. A., Olson, S. T., and Singh, P. *Arch. Biochem. Biophys.* 273:375-388 (1989)]. The enzyme was diluted into HBSA prior to assay in which the final concentration was 0.25 nM.

[0718] Recombinant Tissue Plasminogen Activator (rt-PA) Assay

[0719] rt-PA catalytic activity was determined using the substrate, Pefachrome t-PA ( $CH_3SO_2$ -D-hexahydrotyrosine-glycyl-L-arginine-p-nitroaniline, obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 500 micromolar (about 3-times  $K_m$ ). Human rt-PA (Activase®) was obtained from Genentech Inc. The enzyme was reconstituted in deionized water and diluted into HBSA prior to the assay in which the final concentration was 1.0 nM.

[0720] Plasmin Assay

[0721] Plasmin catalytic activity was determined using the chromogenic substrate, S-2366 [L-pyroglutamyl-L-prolyl-L-arginine-p-nitroaniline hydrochloride], which was obtained from DiaPharma group. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 300 micromolar (about 2.5-times  $K_m$ ). Purified human plasmin was obtained from Enzyme Research Laboratories, Inc. The enzyme was diluted into HBSA prior to assay in which the final concentration was 1.0 nM.

[0722] Activated Protein C (aPC) Assay

[0723] aPC catalytic activity was determined using the chromogenic substrate, Pefachrome PC ( $\delta$ -carbobenzoyloxy-D-lysine-L-prolyl-L-arginine-p-nitroaniline dihydrochloride), obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 400 micromolar (about 3-times  $K_m$ ). Purified human aPC was obtained from Hematologic Technologies, Inc. The enzyme was diluted into HBSA prior to assay in which the final concentration was 1.0 nM.

[0724] Chymotrypsin Assay

[0725] Chymotrypsin catalytic activity was determined using the chromogenic substrate, S-2586 (methoxy-succinyl-L-arginine-L-prolyl-L-tyrosyl-p-nitroanilide), which was obtained from DiaPharma Group. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 100 micromolar (about 9-times  $K_m$ ). Purified (3X-crystallized; CDI) bovine pancreatic alpha-chymotrypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA prior to assay in which the final concentration was 0.5 nM.

[0726] Trypsin Assay

[0727] Trypsin catalytic activity was determined using the chromogenic substrate, S-2222 (benzoyl-L-isoleucine-L-glutamic acid-[ $\gamma$ -methyl ester]-L-arginine-p-nitroanilide), which was obtained from DiaPharma Group. The

substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 250 micromolar (about 4-times Km). Purified (3X-crystallized; TRL3) bovine pancreatic trypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted

into HBSA prior to assay in which the final concentration was 0.5 nM.

[0728] Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

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<301> AUTHORS: O'Brien, T.J. and Tanimoto, H.
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gac ccc cac tgg gtc ctc acg gca gcc cac tgc ttc agg aaa cat acc	1013
Asp Pro His Trp Val Leu Thr Ala Ala His Cys Phe Arg Lys His Thr	
240 245 250	
gat gtg ttc aac tgg aag gtg cgg gca ggc tca gac aaa ctg ggc agc	1061
Asp Val Phe Asn Trp Lys Val Arg Ala Gly Ser Asp Lys Leu Gly Ser	
255 260 265	
ttc cca tcc ctg gct gtg gcc aag atc atc atc att gaa ttc aac ccc	1109
Phe Pro Ser Leu Ala Val Ala Lys Ile Ile Ile Ile Glu Phe Asn Pro	
270 275 280	

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atg tac ccc aaa gac aat gac atc gcc ctc atg aag ctg cag ttc cca 1157
Met Tyr Pro Lys Asp Asn Asp Ile Ala Leu Met Lys Leu Gln Phe Pro
    285                290                295

ctc act ttc tca ggc aca gtc agg ctc atc tgt ctg ccc ttc ttt gat 1205
Leu Thr Phe Ser Gly Thr Val Arg Leu Ile Cys Leu Pro Phe Phe Asp
300                305                310                315

gag gag ctc act cca gcc acc cca ctc tgg atc att gga tgg ggc ttt 1253
Glu Glu Leu Thr Pro Ala Thr Pro Leu Trp Ile Ile Gly Trp Gly Phe
                320                325                330

acg aag cag aat gga ggg aag atg tct gac ata ctg ctg cag gcg tca 1301
Thr Lys Gln Asn Gly Gly Lys Met Ser Asp Ile Leu Leu Gln Ala Ser
                335                340                345

gtc cag gtc att gac agc aca cgg tgc aat gca gac gat gcg tac cag 1349
Val Gln Val Ile Asp Ser Thr Arg Cys Asn Ala Asp Asp Ala Tyr Gln
    350                355                360

ggg gaa gtc acc gag aag atg atg tgt gca ggc atc ccg gaa ggg ggt 1397
Gly Glu Val Thr Glu Lys Met Met Cys Ala Gly Ile Pro Glu Gly Gly
    365                370                375

gtg gac acc tgc cag ggt gac agt ggt ggg ccc ctg atg tac caa tct 1445
Val Asp Thr Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Tyr Gln Ser
380                385                390                395

gac cag tgg cat gtg gtg ggc atc gtt agc tgg ggc tat ggc tgc ggg 1493
Asp Gln Trp His Val Val Gly Ile Val Ser Trp Gly Tyr Gly Cys Gly
    400                405                410

ggc ccg agc acc cca gga gta tac acc aag gtc tca gcc tat ctc aac 1541
Gly Pro Ser Thr Pro Gly Val Tyr Thr Lys Val Ser Ala Tyr Leu Asn
    415                420                425

tgg atc tac aat gtc tgg aag gct gag ctg taa tgetgctgcc cctttgcagt 1594
Trp Ile Tyr Asn Val Trp Lys Ala Glu Leu *
    430                435

gctgggagcc gcttccttcc tgccctgcc acctggggat cccccaagt cagacacaga 1654

gcaagagtcc ccttggttac acccctctgc ccacagcctc agcatttctt ggagcagcaa 1714

agggcctcaa ttcctgtaag agaccctcgc agcccagagg cgcccagagg aagtcagcag 1774

ccctagctcg gccacacttg gtgtccag catcccaggg agagacacag cccactgaac 1834

aaggtctcag gggatttgct aagccaagaa ggaactttcc cacactactg aatggaagca 1894

ggctgtcttg taaaagccca gatcactgtg ggctggagag gagaaggaaa gggctgcgc 1954

cagccctgtc cgtcttcacc catcccgaag cctactagag caagaaacca gttgtaatat 2014

aaaatgcact gccctactgt tggtatgact accgttacct actggtgtca ttgttattac 2074

agctatggcc actattatta aagagctgtg taacaaaaaa aaaaaaaaaa aaaaaaaaaa 2134

aaa 2137
    
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<210> SEQ ID NO 4  
 <211> LENGTH: 437  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo Sapien

<400> SEQUENCE: 4

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Met Leu Gln Asp Pro Asp Ser Asp Gln Pro Leu Asn Ser Leu Asp Val
 1                5                10                15

Lys Pro Leu Arg Lys Pro Arg Ile Pro Met Glu Thr Phe Arg Lys Val
                20                25                30

Gly Ile Pro Ile Ile Ile Ala Leu Leu Ser Leu Ala Ser Ile Ile Ile
 35                40                45
    
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Val Val Val Leu Ile Lys Val Ile Leu Asp Lys Tyr Tyr Phe Leu Cys  
 50 55 60

Gly Gln Pro Leu His Phe Ile Pro Arg Lys Gln Leu Cys Asp Gly Glu  
 65 70 75 80

Leu Asp Cys Pro Leu Gly Glu Asp Glu Glu His Cys Val Lys Ser Phe  
 85 90 95

Pro Glu Gly Pro Ala Val Ala Val Arg Leu Ser Lys Asp Arg Ser Thr  
 100 105 110

Leu Gln Val Leu Asp Ser Ala Thr Gly Asn Trp Phe Ser Ala Cys Phe  
 115 120 125

Asp Asn Phe Thr Glu Ala Leu Ala Glu Thr Ala Cys Arg Gln Met Gly  
 130 135 140

Tyr Ser Ser Lys Pro Thr Phe Arg Ala Val Glu Ile Gly Pro Asp Gln  
 145 150 155 160

Asp Leu Asp Val Val Glu Ile Thr Glu Asn Ser Gln Glu Leu Arg Met  
 165 170 175

Arg Asn Ser Ser Gly Pro Cys Leu Ser Gly Ser Leu Val Ser Leu His  
 180 185 190

Cys Leu Ala Cys Gly Lys Ser Leu Lys Thr Pro Arg Val Val Gly Gly  
 195 200 205

Glu Glu Ala Ser Val Asp Ser Trp Pro Trp Gln Val Ser Ile Gln Tyr  
 210 215 220

Asp Ile Gln His Val Cys Gly Gly Ser Ile Leu Asp Pro His Trp Val  
 225 230 235 240

Leu Thr Ala Ala His Cys Phe Arg Lys His Thr Asp Val Phe Asn Trp  
 245 250 255

Lys Val Arg Ala Gly Ser Asp Lys Leu Gly Ser Phe Pro Ser Leu Ala  
 260 265 270

Val Ala Lys Ile Ile Ile Ile Glu Phe Asn Pro Met Tyr Pro Lys Asp  
 275 280 285

Asn Asp Ile Ala Leu Met Lys Leu Gln Phe Pro Leu Thr Phe Ser Gly  
 290 295 300

Thr Val Arg Leu Ile Cys Leu Pro Phe Phe Asp Glu Glu Leu Thr Pro  
 305 310 315 320

Ala Thr Pro Leu Trp Ile Ile Gly Trp Gly Phe Thr Lys Gln Asn Gly  
 325 330 335

Gly Lys Met Ser Asp Ile Leu Leu Gln Ala Ser Val Gln Val Ile Asp  
 340 345 350

Ser Thr Arg Cys Asn Ala Asp Asp Ala Tyr Gln Gly Glu Val Thr Glu  
 355 360 365

Lys Met Met Cys Ala Gly Ile Pro Glu Gly Gly Val Asp Thr Cys Gln  
 370 375 380

Gly Asp Ser Gly Gly Pro Leu Met Tyr Gln Ser Asp Gln Trp His Val  
 385 390 395 400

Val Gly Ile Val Ser Trp Gly Tyr Gly Cys Gly Gly Pro Ser Thr Pro  
 405 410 415

Gly Val Tyr Thr Lys Val Ser Ala Tyr Leu Asn Trp Ile Tyr Asn Val  
 420 425 430

Trp Lys Ala Glu Leu  
 435

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<210> SEQ ID NO 5
<211> LENGTH: 708
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(708)
<223> OTHER INFORMATION: MTSP4 protease domain cDNA

<400> SEQUENCE: 5

att gtt ggt gga gct gtg tcc tcc gag ggt gag tgg cca tgg cag gcc      48
Ile Val Gly Gly Ala Val Ser Ser Glu Gly Glu Trp Pro Trp Gln Ala
  1             5             10             15

agc ctc cag gtt cgg ggt cga cac atc tgt ggg ggg gcc ctc atc gct      96
Ser Leu Gln Val Arg Gly Arg His Ile Cys Gly Gly Ala Leu Ile Ala
  20             25             30

gac cgc tgg gtg ata aca gct gcc cac tgc ttc cag gag gac agc atg      144
Asp Arg Trp Val Ile Thr Ala Ala His Cys Phe Gln Glu Asp Ser Met
  35             40             45

gcc tcc acg gtg ctg tgg acc gtg ttc ctg ggc aag gtg tgg cag aac      192
Ala Ser Thr Val Leu Trp Thr Val Phe Leu Gly Lys Val Trp Gln Asn
  50             55             60

tcg cgc tgg oct gga gag gtg tcc ttc aag gtg agc cgc ctg ctc ctg      240
Ser Arg Trp Pro Gly Glu Val Ser Phe Lys Val Ser Arg Leu Leu Leu
  65             70             75

cac ccg tac cac gaa gag gac agc cat gac tac gac gtg gcg ctg ctg      288
His Pro Tyr His Glu Glu Asp Ser His Asp Tyr Asp Val Ala Leu Leu
  85             90             95

cag ctc gac cac ccg gtg gtg cgc tcg gcc gcc gtg cgc ccc gtc tgc      336
Gln Leu Asp His Pro Val Val Arg Ser Ala Ala Val Arg Pro Val Cys
  100            105            110

ctg ccc gcg cgc tcc cac ttc ttc gag ccc ggc ctg cac tgc tgg att      384
Leu Pro Ala Arg Ser His Phe Phe Glu Pro Gly Leu His Cys Trp Ile
  115            120            125

acg ggc tgg ggc gcc ttg cgc gag ggc ggc ccc atc agc aac gct ctg      432
Thr Gly Trp Gly Ala Leu Arg Glu Gly Gly Pro Ile Ser Asn Ala Leu
  130            135            140

cag aaa gtg gat gtg cag ttg atc cca cag gac ctg tgc agc gag gtc      480
Gln Lys Val Asp Val Gln Leu Ile Pro Gln Asp Leu Cys Ser Glu Val
  145            150            155

tat cgc tac cag gtg acg cca cgc atg ctg tgt gcc ggc tac cgc aag      528
Tyr Arg Tyr Gln Val Thr Pro Arg Met Leu Cys Ala Gly Tyr Arg Lys
  165            170            175

ggc aag aag gat gcc tgt cag ggt gac tca ggt ggt ccg ctg gtg tgc      576
Gly Lys Lys Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
  180            185            190

aag gca ctc agt ggc cgc tgg ttc ctg gcg ggg ctg gtc agc tgg ggc      624
Lys Ala Leu Ser Gly Arg Trp Phe Leu Ala Gly Leu Val Ser Trp Gly
  195            200            205

ctg ggc tgt ggc cgg cct aac tac ttc ggc gtc tac acc cgc atc aca      672
Leu Gly Cys Gly Arg Pro Asn Tyr Phe Gly Val Tyr Thr Arg Ile Thr
  210            215            220

ggt gtg atc agc tgg atc cag caa gtg gtg acc tga                        708
Gly Val Ile Ser Trp Ile Gln Gln Val Val Thr *
  225            230            235

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<210> SEQ ID NO 6
<211> LENGTH: 235
<212> TYPE: PRT

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<213> ORGANISM: Homo Sapien

<400> SEQUENCE: 6

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Ile Val Gly Gly Ala Val Ser Ser Glu Gly Glu Trp Pro Trp Gln Ala
 1          5          10          15
Ser Leu Gln Val Arg Gly Arg His Ile Cys Gly Gly Ala Leu Ile Ala
          20          25          30
Asp Arg Trp Val Ile Thr Ala Ala His Cys Phe Gln Glu Asp Ser Met
          35          40          45
Ala Ser Thr Val Leu Trp Thr Val Phe Leu Gly Lys Val Trp Gln Asn
          50          55          60
Ser Arg Trp Pro Gly Glu Val Ser Phe Lys Val Ser Arg Leu Leu Leu
65          70          75          80
His Pro Tyr His Glu Glu Asp Ser His Asp Tyr Asp Val Ala Leu Leu
          85          90          95
Gln Leu Asp His Pro Val Val Arg Ser Ala Ala Val Arg Pro Val Cys
          100          105          110
Leu Pro Ala Arg Ser His Phe Phe Glu Pro Gly Leu His Cys Trp Ile
          115          120          125
Thr Gly Trp Gly Ala Leu Arg Glu Gly Gly Pro Ile Ser Asn Ala Leu
130          135          140
Gln Lys Val Asp Val Gln Leu Ile Pro Gln Asp Leu Cys Ser Glu Val
145          150          155          160
Tyr Arg Tyr Gln Val Thr Pro Arg Met Leu Cys Ala Gly Tyr Arg Lys
          165          170          175
Gly Lys Lys Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
          180          185          190
Lys Ala Leu Ser Gly Arg Trp Phe Leu Ala Gly Leu Val Ser Trp Gly
          195          200          205
Leu Gly Cys Gly Arg Pro Asn Tyr Phe Gly Val Tyr Thr Arg Ile Thr
210          215          220
Gly Val Ile Ser Trp Ile Gln Gln Val Val Thr
225          230          235

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<210> SEQ ID NO 7

<211> LENGTH: 3104

<212> TYPE: DNA

<213> ORGANISM: Homo Sapien

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (33)...(2441)

<223> OTHER INFORMATION: cDNA encoding :MTSP4-L (long form) splice variant

<400> SEQUENCE: 7

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tcatcggcca gaggggtgac agtgagcaga ag atg ccc gtg gcc gag gcc ccc      53
          Met Pro Val Ala Glu Ala Pro
          1          5
cag gtg gct ggc ggg cag ggg gac gga ggt gat ggc gag gaa gcg gag      101
Gln Val Ala Gly Gly Gln Gly Asp Gly Gly Asp Gly Glu Glu Ala Glu
          10          15          20
ccg gag ggg atg ttc aag gcc tgt gag gac tcc aag aga aaa gcc cgg      149
Pro Glu Gly Met Phe Lys Ala Cys Glu Asp Ser Lys Arg Lys Ala Arg
          25          30          35
ggc tac ctc cgc ctg gtg ccc ctg ttt gtg ctg ctg gcc ctg ctc gtg      197
Gly Tyr Leu Arg Leu Val Pro Leu Phe Val Leu Leu Ala Leu Leu Val

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40	45	50	55	
ctg gct tgc gcg ggg gtg cta ctc tgg tat ttc cta ggg tac aag gcg Leu Ala Ser Ala Gly Val Leu Leu Trp Tyr Phe Leu Gly Tyr Lys Ala 60 65 70				245
gag gtg atg gtc agc cag gtg tac tca ggc agt ctg cgt gta ctc aat Glu Val Met Val Ser Gln Val Tyr Ser Gly Ser Leu Arg Val Leu Asn 75 80 85				293
cgc cac ttc tcc cag gat ctt acc cgc cgg gaa tct agt gcc ttc cgc Arg His Phe Ser Gln Asp Leu Thr Arg Arg Glu Ser Ser Ala Phe Arg 90 95 100				341
agt gaa acc gcc aaa gcc cag aag atg ctc aag gag ctc atc acc agc Ser Glu Thr Ala Lys Ala Gln Lys Met Leu Lys Glu Leu Ile Thr Ser 105 110 115				389
acc cgc ctg gga act tac tac aac tcc agc tcc gtc tat tcc ttt ggg Thr Arg Leu Gly Thr Tyr Tyr Asn Ser Ser Ser Val Tyr Ser Phe Gly 120 125 130 135				437
gag gga ccc ctc acc tgc ttc ttc tgg ttc att ctc caa atc ccc gag Glu Gly Pro Leu Thr Cys Phe Phe Trp Phe Ile Leu Gln Ile Pro Glu 140 145 150				485
cac cgc cgg ctg atg ctg agc ccc gag gtg gtg cag gca ctg ctg gtg His Arg Arg Leu Met Leu Ser Pro Glu Val Val Gln Ala Leu Leu Val 155 160 165				533
gag gag ctg ctg tcc aca gtc aac agc tcg gct gcc gtc ccc tac agg Glu Glu Leu Leu Ser Thr Val Asn Ser Ser Ala Ala Val Pro Tyr Arg 170 175 180				581
gcc gag tac gaa gtg gac ccc gag ggc cta gtg atc ctg gaa gcc agt Ala Glu Tyr Glu Val Asp Pro Glu Gly Leu Val Ile Leu Glu Ala Ser 185 190 195				629
gtg aaa gac ata gct gca ttg aat tcc acg ctg ggt tgt tac cgc tac Val Lys Asp Ile Ala Ala Leu Asn Ser Thr Leu Gly Cys Tyr Arg Tyr 200 205 210 215				677
agc tac gtg ggc cag ggc cag gtc ctc cgg ctg aag ggg cct gac cac Ser Tyr Val Gly Gln Gly Gln Val Leu Arg Leu Lys Gly Pro Asp His 220 225 230				725
ctg gcc tcc agc tgc ctg tgg cac ctg cag ggc ccc aag gac ctc atg Leu Ala Ser Ser Cys Leu Trp His Leu Gln Gly Pro Lys Asp Leu Met 235 240 245				773
ctc aaa ctc cgg ctg gag tgg acg ctg gca gag tgc cgg gac cga ctg Leu Lys Leu Arg Leu Glu Trp Thr Leu Ala Glu Cys Arg Asp Arg Leu 250 255 260				821
gcc atg tat gac gtg gcc ggg ccc ctg gag aag agg ctc atc acc tcg Ala Met Tyr Asp Val Ala Gly Pro Leu Glu Lys Arg Leu Ile Thr Ser 265 270 275				869
gtg tac ggc tgc agc cgc cag gag ccc gtg gtg gag gtt ctg gcg tcg Val Tyr Gly Cys Ser Arg Gln Glu Pro Val Val Glu Val Leu Ala Ser 280 285 290 295				917
ggg gcc atc atg gcg gtc gtc tgg aag aag ggc ctg cac agc tac tac Gly Ala Ile Met Ala Val Val Trp Lys Lys Gly Leu His Ser Tyr Tyr 300 305 310				965
gac ccc ttc gtg ctc tcc gtg cag ccg gtg gtc ttc cag gcc tgt gaa Asp Pro Phe Val Leu Ser Val Gln Pro Val Val Phe Gln Ala Cys Glu 315 320 325				1013
gtg aac ctg acg ctg gac aac agg ctc gac tcc cag ggc gtc ctc agc Val Asn Leu Thr Leu Asp Asn Arg Leu Asp Ser Gln Gly Val Leu Ser 330 335 340				1061
acc ccg tac ttc ccc agc tac tac tcg ccc caa acc cac tgc tcc tgg Thr Pro Tyr Phe Pro Ser Tyr Tyr Ser Pro Gln Thr His Cys Ser Trp				1109

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345	350	355	
cac ctc acg gtg ccc tct ctg gac tac ggc ttg gcc ctc tgg ttt gat His Leu Thr Val Pro Ser Leu Asp Tyr Gly Leu Ala Leu Trp Phe Asp 360 365 370 375			1157
gcc tat goa ctg agg agg cag aag tat gat ttg ccg tgc acc cag ggc Ala Tyr Ala Leu Arg Arg Gln Lys Tyr Asp Leu Pro Cys Thr Gln Gly 380 385 390			1205
cag tgg acg atc cag aac agg agg ctg tgt ggc ttg cgc atc ctg cag Gln Trp Thr Ile Gln Asn Arg Arg Leu Cys Gly Leu Arg Ile Leu Gln 395 400 405			1253
ccc tac gcc gag agg atc ccc gtg gtg gcc acg gcc ggg atc acc atc Pro Tyr Ala Glu Arg Ile Pro Val Val Ala Thr Ala Gly Ile Thr Ile 410 415 420			1301
aac ttc acc tcc cag atc tcc ctc acc ggg ccc ggt gtg cgg gtg cac Asn Phe Thr Ser Gln Ile Ser Leu Thr Gly Pro Gly Val Arg Val His 425 430 435			1349
tat gcc ttg tac aac cag tcg gac ccc tgc cct gga gag ttc ctc tgt Tyr Gly Leu Tyr Asn Gln Ser Asp Pro Cys Pro Gly Glu Phe Leu Cys 440 445 450 455			1397
tct gtg aat gga ctc tgt gtc cct gcc tgt gat ggg gtc aag gac tgc Ser Val Asn Gly Leu Cys Val Pro Ala Cys Asp Gly Val Lys Asp Cys 460 465 470			1445
ccc aac gcc ctg gat gag aga aac tgc gtt tgc aga gcc aca ttc cag Pro Asn Gly Leu Asp Glu Arg Asn Cys Val Cys Arg Ala Thr Phe Gln 475 480 485			1493
tgc aaa gag gac agc aca tgc atc tca ctg ccc aag gtc tgt gat ggg Cys Lys Glu Asp Ser Thr Cys Ile Ser Leu Pro Lys Val Cys Asp Gly 490 495 500			1541
cag cct gat tgt ctc aac ggc agc gac gaa gag cag tgc cag gaa ggg Gln Pro Asp Cys Leu Asn Gly Ser Asp Glu Glu Gln Cys Gln Glu Gly 505 510 515			1589
gtg cca tgt ggg aca ttc acc ttc cag tgt gag gac cgg agc tgc gtg Val Pro Cys Gly Thr Phe Thr Phe Gln Cys Glu Asp Arg Ser Cys Val 520 525 530 535			1637
aag aag ccc aac ccg cag tgt gat ggg cgg ccc gac tgc agg gac ggc Lys Lys Pro Asn Pro Gln Cys Asp Gly Arg Pro Asp Cys Arg Asp Gly 540 545 550			1685
tcg gat gag gag cac tgt gaa tgt gcc ctc cag gcc ccc tcc agc cgc Ser Asp Glu Glu His Cys Glu Cys Gly Leu Gln Gly Pro Ser Ser Arg 555 560 565			1733
att gtt ggt gga gct gtg tcc tcc gag ggt gag tgg cca tgg cag gcc Ile Val Gly Gly Ala Val Ser Ser Glu Gly Glu Trp Pro Trp Gln Ala 570 575 580			1781
agc ctc cag gtt cgg ggt cga cac atc tgt ggg ggg gcc ctc atc gct Ser Leu Gln Val Arg Gly Arg His Ile Cys Gly Gly Ala Leu Ile Ala 585 590 595			1829
gac cgc tgg gtg ata aca gct gcc cac tgc ttc cag gag gac agc atg Asp Arg Trp Val Ile Thr Ala Ala His Cys Phe Gln Glu Asp Ser Met 600 605 610 615			1877
gcc tcc acg gtg ctg tgg acc gtg ttc ctg ggc aag gtg tgg cag aac Ala Ser Thr Val Leu Trp Thr Val Phe Leu Gly Lys Val Trp Gln Asn 620 625 630			1925
tcg cgc tgg cct gga gag gtg tcc ttc aag gtg agc cgc ctg ctc ctg Ser Arg Trp Pro Gly Glu Val Ser Phe Lys Val Ser Arg Leu Leu Leu 635 640 645			1973
cac ccg tac cac gaa gag gac agc cat gac tac gac gtg cgc ctg ctg His Pro Tyr His Glu Glu Asp Ser His Asp Tyr Asp Val Ala Leu Leu 650 655 660 665			2021

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650	655	660	
cag ctc gac cac ccg gtg gtg cgc tcg gcc gcc gtg cgc ccc gtc tgc			2069
Gln Leu Asp His Pro Val Val Arg Ser Ala Ala Val Arg Pro Val Cys			
665	670	675	
ctg ccc gcg cgc tcc cac ttc ttc gag ccc ggc ctg cac tgc tgg att			2117
Leu Pro Ala Arg Ser His Phe Phe Glu Pro Gly Leu His Cys Trp Ile			
680	685	690	695
acg ggc tgg ggc gcc ttg cgc gag ggc ggc ccc atc agc aac gct ctg			2165
Thr Gly Trp Gly Ala Leu Arg Glu Gly Gly Pro Ile Ser Asn Ala Leu			
	700	705	710
cag aaa gtg gat gtg cag ttg atc cca cag gac ctg tgc agc gag gtc			2213
Gln Lys Val Asp Val Gln Leu Ile Pro Gln Asp Leu Cys Ser Glu Val			
	715	720	725
tat cgc tac cag gtg acg cca cgc atg ctg tgt gcc ggc tac cgc aag			2261
Tyr Arg Tyr Gln Val Thr Pro Arg Met Leu Cys Ala Gly Tyr Arg Lys			
	730	735	740
ggc aag aag gat gcc tgt cag ggt gac tca ggt ggt ccg ctg gtg tgc			2309
Gly Lys Lys Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys			
	745	750	755
aag gca ctc agt ggc cgc tgg ttc ctg gcg ggg ctg gtc agc tgg ggc			2357
Lys Ala Leu Ser Gly Arg Trp Phe Leu Ala Gly Leu Val Ser Trp Gly			
	760	765	770
ctg ggc tgt ggc cgc cct aac tac ttc ggc gtc tac acc cgc atc aca			2405
Leu Gly Cys Gly Arg Pro Asn Tyr Phe Gly Val Tyr Thr Arg Ile Thr			
	780	785	790
ggt gtg atc agc tgg atc cag caa gtg gtg acc tga ggaactgcc			2451
Gly Val Ile Ser Trp Ile Gln Gln Val Val Thr *			
	795	800	
ccctgcaaag cagggccac ctctggact cagagagccc agggcaactg ccaagcaggg			2511
ggacaagtat totggcgggg ggtgggggag agagcaggcc ctgtggtggc aggaggggca			2571
tcttgtttcg tccctgatgt ctgtccagta tggcaggagg atgagaagtg ccagcagttg			2631
ggggtaacaag cgctccctga ggaccaggc ccacaccag ccottttgcc tccaattct			2691
ctctctccg tcccttct ccaactgtgc ctaatgcaag gcagtggctc agcagcaaga			2751
atgctggttc tacatcccga ggagtgtctg aggtgcgccc cactctgtac agaggctgtt			2811
tgggcagcct tgcctccaga gagcagattc cagcttcgga agccctggt ctaacttggg			2871
atctgggaat ggaaggtgct cccatcggag gggaccctca gagccctgga gactgccagg			2931
tgggcctgct gccactgtaa gccaaaaggt ggggaagtcc tgactccagg gtccttgccc			2991
caccctgcc tgccacctgg gccctcacag cccagacct cactgggagg tgagctcagg			3051
tgccctttgg aataaagctg cctgatgcaa aaaaaaaaaa aaaaaaaaaa aaa			3104
<p>&lt;210&gt; SEQ ID NO 8                  &lt;211&gt; LENGTH: 802                  &lt;212&gt; TYPE: PRT                  &lt;213&gt; ORGANISM: Homo Sapien</p>			
<p>&lt;400&gt; SEQUENCE: 8</p>			
Met Pro Val Ala Glu Ala Pro Gln Val Ala Gly Gly Gln Gly Asp Gly			
1	5	10	15
Gly Asp Gly Glu Glu Ala Glu Pro Glu Gly Met Phe Lys Ala Cys Glu			
	20	25	30
Asp Ser Lys Arg Lys Ala Arg Gly Tyr Leu Arg Leu Val Pro Leu Phe			
	35	40	45

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Val Leu Leu Ala Leu Leu Val Leu Ala Ser Ala Gly Val Leu Leu Trp  
 50 55 60

Tyr Phe Leu Gly Tyr Lys Ala Glu Val Met Val Ser Gln Val Tyr Ser  
 65 70 75 80

Gly Ser Leu Arg Val Leu Asn Arg His Phe Ser Gln Asp Leu Thr Arg  
 85 90 95

Arg Glu Ser Ser Ala Phe Arg Ser Glu Thr Ala Lys Ala Gln Lys Met  
 100 105 110

Leu Lys Glu Leu Ile Thr Ser Thr Arg Leu Gly Thr Tyr Tyr Asn Ser  
 115 120 125

Ser Ser Val Tyr Ser Phe Gly Glu Gly Pro Leu Thr Cys Phe Phe Trp  
 130 135 140

Phe Ile Leu Gln Ile Pro Glu His Arg Arg Leu Met Leu Ser Pro Glu  
 145 150 155 160

Val Val Gln Ala Leu Leu Val Glu Glu Leu Leu Ser Thr Val Asn Ser  
 165 170 175

Ser Ala Ala Val Pro Tyr Arg Ala Glu Tyr Glu Val Asp Pro Glu Gly  
 180 185 190

Leu Val Ile Leu Glu Ala Ser Val Lys Asp Ile Ala Ala Leu Asn Ser  
 195 200 205

Thr Leu Gly Cys Tyr Arg Tyr Ser Tyr Val Gly Gln Gly Gln Val Leu  
 210 215 220

Arg Leu Lys Gly Pro Asp His Leu Ala Ser Ser Cys Leu Trp His Leu  
 225 230 235 240

Gln Gly Pro Lys Asp Leu Met Leu Lys Leu Arg Leu Glu Trp Thr Leu  
 245 250 255

Ala Glu Cys Arg Asp Arg Leu Ala Met Tyr Asp Val Ala Gly Pro Leu  
 260 265 270

Glu Lys Arg Leu Ile Thr Ser Val Tyr Gly Cys Ser Arg Gln Glu Pro  
 275 280 285

Val Val Glu Val Leu Ala Ser Gly Ala Ile Met Ala Val Val Trp Lys  
 290 295 300

Lys Gly Leu His Ser Tyr Tyr Asp Pro Phe Val Leu Ser Val Gln Pro  
 305 310 315 320

Val Val Phe Gln Ala Cys Glu Val Asn Leu Thr Leu Asp Asn Arg Leu  
 325 330 335

Asp Ser Gln Gly Val Leu Ser Thr Pro Tyr Phe Pro Ser Tyr Tyr Ser  
 340 345 350

Pro Gln Thr His Cys Ser Trp His Leu Thr Val Pro Ser Leu Asp Tyr  
 355 360 365

Gly Leu Ala Leu Trp Phe Asp Ala Tyr Ala Leu Arg Arg Gln Lys Tyr  
 370 375 380

Asp Leu Pro Cys Thr Gln Gly Gln Trp Thr Ile Gln Asn Arg Arg Leu  
 385 390 395 400

Cys Gly Leu Arg Ile Leu Gln Pro Tyr Ala Glu Arg Ile Pro Val Val  
 405 410 415

Ala Thr Ala Gly Ile Thr Ile Asn Phe Thr Ser Gln Ile Ser Leu Thr  
 420 425 430

Gly Pro Gly Val Arg Val His Tyr Gly Leu Tyr Asn Gln Ser Asp Pro  
 435 440 445

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Cys Pro Gly Glu Phe Leu Cys Ser Val Asn Gly Leu Cys Val Pro Ala  
 450 455 460

Cys Asp Gly Val Lys Asp Cys Pro Asn Gly Leu Asp Glu Arg Asn Cys  
 465 470 475 480

Val Cys Arg Ala Thr Phe Gln Cys Lys Glu Asp Ser Thr Cys Ile Ser  
 485 490 495

Leu Pro Lys Val Cys Asp Gly Gln Pro Asp Cys Leu Asn Gly Ser Asp  
 500 505 510

Glu Glu Gln Cys Gln Glu Gly Val Pro Cys Gly Thr Phe Thr Phe Gln  
 515 520 525

Cys Glu Asp Arg Ser Cys Val Lys Lys Pro Asn Pro Gln Cys Asp Gly  
 530 535 540

Arg Pro Asp Cys Arg Asp Gly Ser Asp Glu Glu His Cys Glu Cys Gly  
 545 550 555 560

Leu Gln Gly Pro Ser Ser Arg Ile Val Gly Gly Ala Val Ser Ser Glu  
 565 570 575

Gly Glu Trp Pro Trp Gln Ala Ser Leu Gln Val Arg Gly Arg His Ile  
 580 585 590

Cys Gly Gly Ala Leu Ile Ala Asp Arg Trp Val Ile Thr Ala Ala His  
 595 600 605

Cys Phe Gln Glu Asp Ser Met Ala Ser Thr Val Leu Trp Thr Val Phe  
 610 615 620

Leu Gly Lys Val Trp Gln Asn Ser Arg Trp Pro Gly Glu Val Ser Phe  
 625 630 635 640

Lys Val Ser Arg Leu Leu Leu His Pro Tyr His Glu Glu Asp Ser His  
 645 650 655

Asp Tyr Asp Val Ala Leu Leu Gln Leu Asp His Pro Val Val Arg Ser  
 660 665 670

Ala Ala Val Arg Pro Val Cys Leu Pro Ala Arg Ser His Phe Phe Glu  
 675 680 685

Pro Gly Leu His Cys Trp Ile Thr Gly Trp Gly Ala Leu Arg Glu Gly  
 690 695 700

Gly Pro Ile Ser Asn Ala Leu Gln Lys Val Asp Val Gln Leu Ile Pro  
 705 710 715 720

Gln Asp Leu Cys Ser Glu Val Tyr Arg Tyr Gln Val Thr Pro Arg Met  
 725 730 735

Leu Cys Ala Gly Tyr Arg Lys Gly Lys Lys Asp Ala Cys Gln Gly Asp  
 740 745 750

Ser Gly Gly Pro Leu Val Cys Lys Ala Leu Ser Gly Arg Trp Phe Leu  
 755 760 765

Ala Gly Leu Val Ser Trp Gly Leu Gly Cys Gly Arg Pro Asn Tyr Phe  
 770 775 780

Gly Val Tyr Thr Arg Ile Thr Gly Val Ile Ser Trp Ile Gln Gln Val  
 785 790 795 800

Val Thr

<210> SEQ ID NO 9  
 <211> LENGTH: 2672  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo Sapien  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (33)...(2009)

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<223> OTHER INFORMATION: cDNA encoding: MTSP4-S (short form) splice variant

<400> SEQUENCE: 9

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tcatcggcca gaggggtgac agtgagcaga ag atg ccc gtg gcc gag gcc ccc      53
                               Met Pro Val Ala Glu Ala Pro
                               1           5

cag gtg gct ggc ggg cag ggg gac gga ggt gat ggc gag gaa gcg gag      101
Gln Val Ala Gly Gly Gln Gly Asp Gly Gly Asp Gly Glu Glu Ala Glu
          10           15           20

ccg gag ggg atg ttc aag gcc tgt gag gac tcc aag aga aaa gcc cgg      149
Pro Glu Gly Met Phe Lys Ala Cys Glu Asp Ser Lys Arg Lys Ala Arg
          25           30           35

ggc tac ctc cgc ctg gtg ccc ctg ttt gtg ctg ctg gcc ctg ctc gtg      197
Gly Tyr Leu Arg Leu Val Pro Leu Phe Val Leu Leu Ala Leu Leu Val
          40           45           50           55

ctg gct tcg gcg ggg gtg cta ctc tgg tat ttc cta ggg tac aag gcg      245
Leu Ala Ser Ala Gly Val Leu Leu Trp Tyr Phe Leu Gly Tyr Lys Ala
          60           65           70

gag gtg atg gtc agc cag gtg tac tca ggc agt ctg cgt gta ctc aat      293
Glu Val Met Val Ser Gln Val Tyr Ser Gly Ser Leu Arg Val Leu Asn
          75           80           85

cgc cac ttc tcc cag gat ctt acc cgc cgg gaa tct agt gcc ttc cgc      341
Arg His Phe Ser Gln Asp Leu Thr Arg Arg Glu Ser Ser Ala Phe Arg
          90           95           100

agt gaa acc gcc aaa gcc cag aag atg ctc aag gag ctc atc acc agc      389
Ser Glu Thr Ala Lys Ala Gln Lys Met Leu Lys Glu Leu Ile Thr Ser
          105           110           115

acc cgc ctg gga act tac tac aac tcc agc tcc gtc tat tcc ttt ggg      437
Thr Arg Leu Gly Thr Tyr Tyr Asn Ser Ser Ser Val Tyr Ser Phe Gly
          120           125           130           135

gtg tac ggc tgc agc cgc cag gag ccc gtg gtg gag gtt ctg gcg tcg      485
Val Tyr Gly Cys Ser Arg Gln Glu Pro Val Val Glu Val Leu Ala Ser
          140           145           150

ggg gcc atc atg gcg gtc gtc tgg aag aag ggc ctg cac agc tac tac      533
Gly Ala Ile Met Ala Val Val Trp Lys Lys Gly Leu His Ser Tyr Tyr
          155           160           165

gac ccc ttc gtg ctc tcc gtg cag ccg gtg gtc ttc cag gcc tgt gaa      581
Asp Pro Phe Val Leu Ser Val Gln Pro Val Val Phe Gln Ala Cys Glu
          170           175           180

gtg aac ctg acg ctg gac aac agg ctc gac tcc cag ggc gtc ctc agc      629
Val Asn Leu Thr Leu Asp Asn Arg Leu Asp Ser Gln Gly Val Leu Ser
          185           190           195

acc ccg tac ttc ccc agc tac tac tcg ccc caa acc cac tgc tcc tgg      677
Thr Pro Tyr Phe Pro Ser Tyr Tyr Ser Pro Gln Thr His Cys Ser Trp
          200           205           210           215

cac ctc acg gtg ccc tct ctg gac tac ggc ttg gcc ctc tgg ttt gat      725
His Leu Thr Val Pro Ser Leu Asp Tyr Gly Leu Ala Leu Trp Phe Asp
          220           225           230

gcc tat gca ctg agg agg cag aag tat gat ttg ccg tgc acc cag ggc      773
Ala Tyr Ala Leu Arg Arg Gln Lys Tyr Asp Leu Pro Cys Thr Gln Gly
          235           240           245

cag tgg acg atc cag aac agg agg ctg tgt ggc ttg cgc atc ctg cag      821
Gln Trp Thr Ile Gln Asn Arg Arg Leu Cys Gly Leu Arg Ile Leu Gln
          250           255           260

ccc tac gcc gag agg atc ccc gtg gtg gcc acg gcc ggg atc acc atc      869
Pro Tyr Ala Glu Arg Ile Pro Val Val Ala Thr Ala Gly Ile Thr Ile
          265           270           275
    
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aac ttc acc tcc cag atc tcc ctc acc ggg ccc ggt gtg cgg gtg cac	917
Asn Phe Thr Ser Gln Ile Ser Leu Thr Gly Pro Gly Val Arg Val His	
280 285 290 295	
tat ggc ttg tac aac cag tcg gac ccc tgc cct gga gag ttc ctc tgt	965
Tyr Gly Leu Tyr Asn Gln Ser Asp Pro Cys Pro Gly Glu Phe Leu Cys	
300 305 310	
tct gtg aat gga ctc tgt gtc cct gcc tgt gat ggg gtc aag gac tgc	1013
Ser Val Asn Gly Leu Cys Val Pro Ala Cys Asp Gly Val Lys Asp Cys	
315 320 325	
ccc aac ggc ctg gat gag aga aac tgc gtt tgc aga gcc aca ttc cac	1061
Pro Asn Gly Leu Asp Glu Arg Asn Cys Val Cys Arg Ala Thr Phe Gln	
330 335 340	
tgc aaa gag gac agc aca tgc atc tca ctg ccc aag gtc tgt gat ggg	1109
Cys Lys Glu Asp Ser Thr Cys Ile Ser Leu Pro Lys Val Cys Asp Gly	
345 350 355	
cag cct gat tgt ctc aac ggc agc gac gaa gag cag tgc cag gaa ggg	1157
Gln Pro Asp Cys Leu Asn Gly Ser Asp Glu Gln Cys Gln Glu Gly	
360 365 370 375	
gtg cca tgt ggg aca ttc acc ttc cag tgt gag gac cgg agc tgc gtg	1205
Val Pro Cys Gly Thr Phe Thr Phe Gln Cys Glu Asp Arg Ser Cys Val	
380 385 390	
aag aag ccc aac ccg cag tgt gat ggg cgg ccc gac tgc agg gac ggc	1253
Lys Lys Pro Asn Pro Gln Cys Asp Gly Arg Pro Asp Cys Arg Asp Gly	
395 400 405	
tcg gat gag gag cac tgt gaa tgt ggc ctc cag ggc ccc tcc agc cgc	1301
Ser Asp Glu Glu His Cys Glu Cys Gly Leu Gln Gly Pro Ser Ser Arg	
410 415 420	
att gtt ggt gga gct gtg tcc tcc gag ggt gag tgg cca tgg cag gcc	1349
Ile Val Gly Gly Ala Val Ser Ser Glu Gly Glu Trp Pro Trp Gln Ala	
425 430 435	
agc ctc cag gtt cgg ggt cga cac atc tgt ggg ggg gcc ctc atc gct	1397
Ser Leu Gln Val Arg Gly Arg His Ile Cys Gly Gly Ala Leu Ile Ala	
440 445 450 455	
gac cgc tgg gtg ata aca gct gcc cac tgc ttc cag gag gac agc atg	1445
Asp Arg Trp Val Ile Thr Ala Ala His Cys Phe Gln Glu Asp Ser Met	
460 465 470	
gcc tcc acg gtg ctg tgg acc gtg ttc ctg ggc aag gtg tgg cag aac	1493
Ala Ser Thr Val Leu Trp Thr Val Phe Leu Gly Lys Val Trp Gln Asn	
475 480 485	
tcg cgc tgg cct gga gag gtg tcc ttc aag gtg agc cgc ctg ctc ctg	1541
Ser Arg Trp Pro Gly Glu Val Ser Phe Lys Val Ser Arg Leu Leu Leu	
490 495 500	
cac ccg tac cac gaa gag gac agc cat gac tac gac gtg cgc ctg ctg	1589
His Pro Tyr His Glu Glu Asp Ser His Asp Tyr Asp Val Ala Leu Leu	
505 510 515	
cag ctc gac cac ccg gtg gtg cgc tcg gcc gcc gtg cgc ccc gtc tgc	1637
Gln Leu Asp His Pro Val Val Arg Ser Ala Ala Val Arg Pro Val Cys	
520 525 530 535	
ctg ccc cgg cgc tcc cac ttc ttc gag ccc ggc ctg cac tgc tgg att	1685
Leu Pro Ala Arg Ser His Phe Phe Glu Pro Gly Leu His Cys Trp Ile	
540 545 550	
acg ggc tgg ggc gcc ttg cgc gag ggc gcc ccc atc agc aac gct ctg	1733
Thr Gly Trp Gly Ala Leu Arg Glu Gly Gly Pro Ile Ser Asn Ala Leu	
555 560 565	
cag aaa gtg gat gtg cag ttg atc cca cag gac ctg tgc agc gag gtc	1781
Gln Lys Val Asp Val Gln Leu Ile Pro Gln Asp Leu Cys Ser Glu Val	
570 575 580	

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tat cgc tac cag gtg acg cca cgc atg ctg tgt gcc gcc tac cgc aag      1829
Tyr Arg Tyr Gln Val Thr Pro Arg Met Leu Cys Ala Gly Tyr Arg Lys
      585                590                595

ggc aag aag gat gcc tgt cag ggt gac tca ggt ggt ccg ctg gtg tgc      1877
Gly Lys Lys Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
600                605                610                615

aag gca ctc agt ggc cgc tgg ttc ctg gcg ggg ctg gtc agc tgg ggc      1925
Lys Ala Leu Ser Gly Arg Trp Phe Leu Ala Gly Leu Val Ser Trp Gly
                620                625                630

ctg gcc tgt ggc cgg cct aac tac ttc gcc gtc tac acc cgc atc aca      1973
Leu Gly Cys Gly Arg Pro Asn Tyr Phe Gly Val Tyr Thr Arg Ile Thr
                635                640                645

ggt gtg atc agc tgg atc cag caa gtg gtg acc tga ggaactgccc      2019
Gly Val Ile Ser Trp Ile Gln Gln Val Val Thr *
      650                655

ccctgcaaag cagggcccac ctctggact cagagagccc agggcaactg ccaagcaggg      2079

ggacaagtat tctggcgggg ggtgggggag agagcagggc ctgtggtggc aggaggggca      2139

tcttgtttcg tccctgatgt ctgtccagta tggcaggagg atgagaagtg ccagcagttg      2199

ggggtaacaag cgctccctga ggaccaggc ccacaccag ccottttgcc tccaattct      2259

ctctctcccg tccccttct ccaactgctgc ctaatgcaag gcagtggctc agcagcaaga      2319

atgctgggttc tacatcccga ggagtgtctg aggtgcgccc cactctgtac agaggctgtt      2379

tgggcagcct tgctcccaga gagcagattc cagcttcgga agcccctggt ctaacttggg      2439

atctgggaat ggaaggtgct cccatcggag gggaccctca gagccctgga gactgccagg      2499

tgggcctgct gccactgtaa gccaaaaggt ggggaagtcc tgactccagg gtccttgccc      2559

caccctgccc tgccacctgg gcctcacag cccagaccct cactgggagg tgagctcagc      2619

tgccctttgg aataaagctg cctgatgcaa aaaaaaaaaa aaaaaaaaaa aaa      2672
    
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<210> SEQ ID NO 10
<211> LENGTH: 658
<212> TYPE: PRT
<213> ORGANISM: Homo Sapien
    
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<400> SEQUENCE: 10

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Met Pro Val Ala Glu Ala Pro Gln Val Ala Gly Gly Gln Gly Asp Gly
 1          5          10          15

Gly Asp Gly Glu Glu Ala Glu Pro Glu Gly Met Phe Lys Ala Cys Glu
 20          25          30

Asp Ser Lys Arg Lys Ala Arg Gly Tyr Leu Arg Leu Val Pro Leu Phe
 35          40          45

Val Leu Leu Ala Leu Leu Val Leu Ala Ser Ala Gly Val Leu Leu Trp
 50          55          60

Tyr Phe Leu Gly Tyr Lys Ala Glu Val Met Val Ser Gln Val Tyr Ser
 65          70          75          80

Gly Ser Leu Arg Val Leu Asn Arg His Phe Ser Gln Asp Leu Thr Arg
 85          90          95

Arg Glu Ser Ser Ala Phe Arg Ser Glu Thr Ala Lys Ala Gln Lys Met
 100         105         110

Leu Lys Glu Leu Ile Thr Ser Thr Arg Leu Gly Thr Tyr Tyr Asn Ser
 115         120         125

Ser Ser Val Tyr Ser Phe Gly Val Tyr Gly Cys Ser Arg Gln Glu Pro
    
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130			135			140									
Val	Val	Glu	Val	Leu	Ala	Ser	Gly	Ala	Ile	Met	Ala	Val	Val	Trp	Lys
145					150					155					160
Lys	Gly	Leu	His	Ser	Tyr	Tyr	Asp	Pro	Phe	Val	Leu	Ser	Val	Gln	Pro
			165						170					175	
Val	Val	Phe	Gln	Ala	Cys	Glu	Val	Asn	Leu	Thr	Leu	Asp	Asn	Arg	Leu
			180					185					190		
Asp	Ser	Gln	Gly	Val	Leu	Ser	Thr	Pro	Tyr	Phe	Pro	Ser	Tyr	Tyr	Ser
		195					200					205			
Pro	Gln	Thr	His	Cys	Ser	Trp	His	Leu	Thr	Val	Pro	Ser	Leu	Asp	Tyr
		210				215					220				
Gly	Leu	Ala	Leu	Trp	Phe	Asp	Ala	Tyr	Ala	Leu	Arg	Arg	Gln	Lys	Tyr
225					230					235				240	
Asp	Leu	Pro	Cys	Thr	Gln	Gly	Gln	Trp	Thr	Ile	Gln	Asn	Arg	Arg	Leu
			245						250					255	
Cys	Gly	Leu	Arg	Ile	Leu	Gln	Pro	Tyr	Ala	Glu	Arg	Ile	Pro	Val	Val
			260					265					270		
Ala	Thr	Ala	Gly	Ile	Thr	Ile	Asn	Phe	Thr	Ser	Gln	Ile	Ser	Leu	Thr
		275					280					285			
Gly	Pro	Gly	Val	Arg	Val	His	Tyr	Gly	Leu	Tyr	Asn	Gln	Ser	Asp	Pro
	290					295					300				
Cys	Pro	Gly	Glu	Phe	Leu	Cys	Ser	Val	Asn	Gly	Leu	Cys	Val	Pro	Ala
305					310					315				320	
Cys	Asp	Gly	Val	Lys	Asp	Cys	Pro	Asn	Gly	Leu	Asp	Glu	Arg	Asn	Cys
			325						330					335	
Val	Cys	Arg	Ala	Thr	Phe	Gln	Cys	Lys	Glu	Asp	Ser	Thr	Cys	Ile	Ser
			340					345					350		
Leu	Pro	Lys	Val	Cys	Asp	Gly	Gln	Pro	Asp	Cys	Leu	Asn	Gly	Ser	Asp
		355				360						365			
Glu	Glu	Gln	Cys	Gln	Glu	Gly	Val	Pro	Cys	Gly	Thr	Phe	Thr	Phe	Gln
	370					375					380				
Cys	Glu	Asp	Arg	Ser	Cys	Val	Lys	Lys	Pro	Asn	Pro	Gln	Cys	Asp	Gly
385					390					395				400	
Arg	Pro	Asp	Cys	Arg	Asp	Gly	Ser	Asp	Glu	Glu	His	Cys	Glu	Cys	Gly
			405						410					415	
Leu	Gln	Gly	Pro	Ser	Ser	Arg	Ile	Val	Gly	Gly	Ala	Val	Ser	Ser	Glu
			420					425					430		
Gly	Glu	Trp	Pro	Trp	Gln	Ala	Ser	Leu	Gln	Val	Arg	Gly	Arg	His	Ile
		435					440					445			
Cys	Gly	Gly	Ala	Leu	Ile	Ala	Asp	Arg	Trp	Val	Ile	Thr	Ala	Ala	His
	450					455					460				
Cys	Phe	Gln	Glu	Asp	Ser	Met	Ala	Ser	Thr	Val	Leu	Trp	Thr	Val	Phe
465					470					475				480	
Leu	Gly	Lys	Val	Trp	Gln	Asn	Ser	Arg	Trp	Pro	Gly	Glu	Val	Ser	Phe
			485						490					495	
Lys	Val	Ser	Arg	Leu	Leu	Leu	His	Pro	Tyr	His	Glu	Glu	Asp	Ser	His
			500					505					510		
Asp	Tyr	Asp	Val	Ala	Leu	Leu	Gln	Leu	Asp	His	Pro	Val	Val	Arg	Ser
		515					520					525			
Ala	Ala	Val	Arg	Pro	Val	Cys	Leu	Pro	Ala	Arg	Ser	His	Phe	Phe	Glu
530						535					540				

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Pro Gly Leu His Cys Trp Ile Thr Gly Trp Gly Ala Leu Arg Glu Gly  
 545 550 555 560  
 Gly Pro Ile Ser Asn Ala Leu Gln Lys Val Asp Val Gln Leu Ile Pro  
 565 570 575  
 Gln Asp Leu Cys Ser Glu Val Tyr Arg Tyr Gln Val Thr Pro Arg Met  
 580 585 590  
 Leu Cys Ala Gly Tyr Arg Lys Gly Lys Lys Asp Ala Cys Gln Gly Asp  
 595 600 605  
 Ser Gly Gly Pro Leu Val Cys Lys Ala Leu Ser Gly Arg Trp Phe Leu  
 610 615 620  
 Ala Gly Leu Val Ser Trp Gly Leu Gly Cys Gly Arg Pro Asn Tyr Phe  
 625 630 635 640  
 Gly Val Tyr Thr Arg Ile Thr Gly Val Ile Ser Trp Ile Gln Gln Val  
 645 650 655

Val Thr

<210> SEQ ID NO 11  
 <211> LENGTH: 1656  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo Sapien  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (268)...(1629)  
 <223> OTHER INFORMATION: DNA sequence encoding a transmembrane serine  
 protease (MTSP-6) protein

<400> SEQUENCE: 11

cgccccgggca ggtcagtaac actgtggcct actatctctt ccgtggtgcc atctacattt 60  
 ttgggactcg ggaattatga ctgtttttgg ttaatcgata ctgaatgcgc tttgtgtgga 120  
 ctgtcgaatt tcaaagattt accgtatgac caagatgcac ctgatgctac aagtataaat 180  
 aggggaacaa atgctttctg ttcttctctg gctaaggagg tagagggtgga ggcggagccg 240  
 gatgtcagag gtctctgaaat agtcacc atg ggg gaa aat gat ccg cct gct gtt 294  
 Met Gly Glu Asn Asp Pro Pro Ala Val  
 1 5  
 gaa gcc ccc ttc tca ttc cga tcg ctt ttt ggc ctt gat gat ttg aaa 342  
 Glu Ala Pro Phe Ser Phe Arg Ser Leu Phe Gly Leu Asp Asp Leu Lys  
 10 15 20 25  
 ata agt cct gtt gca cca gat gca gat gct gtt gct gca cag atc ctg 390  
 Ile Ser Pro Val Ala Pro Asp Ala Asp Ala Val Ala Ala Gln Ile Leu  
 30 35 40  
 tca ctg ctg cca ttg aag ttt ttt cca atc atc gtc att ggg atc att 438  
 Ser Leu Leu Pro Leu Lys Phe Phe Pro Ile Ile Val Ile Gly Ile Ile  
 45 50 55  
 gca ttg ata tta gca ctg gcc att ggt ctg ggc atc cac ttc gac tgc 486  
 Ala Leu Ile Leu Ala Leu Ala Ile Gly Leu Gly Ile His Phe Asp Cys  
 60 65 70  
 tca ggg aag tac aga tgt cgc tca tcc ttt aag tgt atc gag ctg ata 534  
 Ser Gly Lys Tyr Arg Cys Arg Ser Ser Phe Lys Cys Ile Glu Leu Ile  
 75 80 85  
 gct cga tgt gac gga gtc tcg gat tgc aaa gac ggg gag gac gag tac 582  
 Ala Arg Cys Asp Gly Val Ser Asp Cys Lys Asp Gly Glu Asp Glu Tyr  
 90 95 100 105  
 cgc tgt gtc cgg gtg ggt ggt cag aat gcc gtg ctc cag gtg ttc aca 630  
 Arg Cys Val Arg Val Gly Gly Gln Asn Ala Val Leu Gln Val Phe Thr  
 110 115 120

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gct gct tcg tgg aag acc atg tgc tcc gat gac tgg aag ggt cac tac	678
Ala Ala Ser Trp Lys Thr Met Cys Ser Asp Asp Trp Lys Gly His Tyr	
125 130 135	
gca aat gtt gcc tgt gcc caa ctg ggt ttc cca agc tat gta agt tca	726
Ala Asn Val Ala Cys Ala Gln Leu Gly Phe Pro Ser Tyr Val Ser Ser	
140 145 150	
gat aac ctc aga gtg agc tcg cta gag ggg cag ttc cgg gag gag ttt	774
Asp Asn Leu Arg Val Ser Ser Leu Glu Gly Gln Phe Arg Glu Glu Phe	
155 160 165	
gtg tcc atc gat cac ctc ttg cca gat gac aag gtg act gca tta cac	822
Val Ser Ile Asp His Leu Leu Pro Asp Asp Lys Val Thr Ala Leu His	
170 175 180 185	
cac tca gta tat gtg agg gag gga tgt gcc tct ggc cac gtg gtt acc	870
His Ser Val Tyr Val Arg Glu Gly Cys Ala Ser Gly His Val Val Thr	
190 195 200	
ttg cag tgc aca gcc tgt ggt cat aga agg ggc tac agc tca cgc atc	918
Leu Gln Cys Thr Ala Cys Gly His Arg Arg Gly Tyr Ser Ser Arg Ile	
205 210 215	
gtg ggt gga aac atg tcc ttg ctc tcg cag tgg ccc tgg cag gcc agc	966
Val Gly Gly Asn Met Ser Leu Leu Ser Gln Trp Pro Trp Gln Ala Ser	
220 225 230	
ctt cag ttc cag ggc tac cac ctg tgc ggg ggc tct gtc atc acg ccc	1014
Leu Gln Phe Gln Gly Tyr His Leu Cys Gly Gly Ser Val Ile Thr Pro	
235 240 245	
ctg tgg atc atc act gct gca cac tgt gtt tat gac ttg tac ctc ccc	1062
Leu Trp Ile Ile Thr Ala Ala His Cys Val Tyr Asp Leu Tyr Leu Pro	
250 255 260 265	
aag tca tgg acc atc cag gtg ggt cta gtt tcc ctg ttg gac aat cca	1110
Lys Ser Trp Thr Ile Gln Val Gly Leu Val Ser Leu Leu Asp Asn Pro	
270 275 280	
gcc cca tcc cac ttg gtg gag aag att gtc tac cac agc aag tac aag	1158
Ala Pro Ser His Leu Val Glu Lys Ile Val Tyr His Ser Lys Tyr Lys	
285 290 295	
cca aag agg ctg ggc aat gac atc gcc ctt atg aag ctg gcc ggg cca	1206
Pro Lys Arg Leu Gly Asn Asp Ile Ala Leu Met Lys Leu Ala Gly Pro	
300 305 310	
ctc acg ttc aat gaa atg atc cag cct gtg tgc ctg ccc aac tct gaa	1254
Leu Thr Phe Asn Glu Met Ile Gln Pro Val Cys Leu Pro Asn Ser Glu	
315 320 325	
gag aac ttc ccc gat gga aaa gtg tgc tgg acg tca gga tgg ggg gcc	1302
Glu Asn Phe Pro Asp Gly Lys Val Cys Trp Thr Ser Gly Trp Gly Ala	
330 335 340 345	
aca gag gat gga ggt gac gcc tcc cct gtc ctg aac cac gcg gcc gtc	1350
Thr Glu Asp Gly Gly Asp Ala Ser Pro Val Leu Asn His Ala Ala Val	
350 355 360	
cct ttg att tcc aac aag atc tgc aac cac agg gac gtg tac ggt ggc	1398
Pro Leu Ile Ser Asn Lys Ile Cys Asn His Arg Asp Val Tyr Gly Gly	
365 370 375	
atc atc tcc ccc tcc atg ctc tgc gcg ggc tac ctg acg ggt ggc gtg	1446
Ile Ile Ser Pro Ser Met Leu Cys Ala Gly Tyr Leu Thr Gly Gly Val	
380 385 390	
gac agc tgc cag ggg gac agc ggg ggg ccc ctg gtg tgt caa gag agg	1494
Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Gln Glu Arg	
395 400 405	
agg ctg tgg aag tta gtg gga gcg acc agc ttt ggc atc ggc tgc gca	1542
Arg Leu Trp Lys Leu Val Gly Ala Thr Ser Phe Gly Ile Gly Cys Ala	
410 415 420 425	

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gag gtg aac aag cct ggg gtg tac acc cgt gtc acc tcc ttc ctg gac      1590
Glu Val Asn Lys Pro Gly Val Tyr Thr Arg Val Thr Ser Phe Leu Asp
                430                      435                      440

tgg atc cac gag cag atg gag aga gac cta aaa acc tga agaggaaggg      1639
Trp Ile His Glu Gln Met Glu Arg Asp Leu Lys Thr *
                445                      450

gataagtagc cacctga                                                    1656

<210> SEQ ID NO 12
<211> LENGTH: 453
<212> TYPE: PRT
<213> ORGANISM: Homo Sapien

<400> SEQUENCE: 12
Met Gly Glu Asn Asp Pro Pro Ala Val Glu Ala Pro Phe Ser Phe Arg
 1                    5                      10                      15
Ser Leu Phe Gly Leu Asp Asp Leu Lys Ile Ser Pro Val Ala Pro Asp
                20                      25                      30
Ala Asp Ala Val Ala Ala Gln Ile Leu Ser Leu Leu Pro Leu Lys Phe
 35                      40                      45
Phe Pro Ile Ile Val Ile Gly Ile Ile Ala Leu Ile Leu Ala Leu Ala
 50                      55                      60
Ile Gly Leu Gly Ile His Phe Asp Cys Ser Gly Lys Tyr Arg Cys Arg
 65                      70                      75                      80
Ser Ser Phe Lys Cys Ile Glu Leu Ile Ala Arg Cys Asp Gly Val Ser
 85                      90                      95
Asp Cys Lys Asp Gly Glu Asp Glu Tyr Arg Cys Val Arg Val Gly Gly
 100                     105                     110
Gln Asn Ala Val Leu Gln Val Phe Thr Ala Ala Ser Trp Lys Thr Met
 115                     120                     125
Cys Ser Asp Asp Trp Lys Gly His Tyr Ala Asn Val Ala Cys Ala Gln
 130                     135                     140
Leu Gly Phe Pro Ser Tyr Val Ser Ser Asp Asn Leu Arg Val Ser Ser
 145                     150                     155                     160
Leu Glu Gly Gln Phe Arg Glu Glu Phe Val Ser Ile Asp His Leu Leu
 165                     170                     175
Pro Asp Asp Lys Val Thr Ala Leu His His Ser Val Tyr Val Arg Glu
 180                     185                     190
Gly Cys Ala Ser Gly His Val Val Thr Leu Gln Cys Thr Ala Cys Gly
 195                     200                     205
His Arg Arg Gly Tyr Ser Ser Arg Ile Val Gly Gly Asn Met Ser Leu
 210                     215                     220
Leu Ser Gln Trp Pro Trp Gln Ala Ser Leu Gln Phe Gln Gly Tyr His
 225                     230                     235                     240
Leu Cys Gly Gly Ser Val Ile Thr Pro Leu Trp Ile Ile Thr Ala Ala
 245                     250                     255
His Cys Val Tyr Asp Leu Tyr Leu Pro Lys Ser Trp Thr Ile Gln Val
 260                     265                     270
Gly Leu Val Ser Leu Leu Asp Asn Pro Ala Pro Ser His Leu Val Glu
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Lys Ile Val Tyr His Ser Lys Tyr Lys Pro Lys Arg Leu Gly Asn Asp
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Gln Pro Val Cys Leu Pro Asn Ser Glu Glu Asn Phe Pro Asp Gly Lys  
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Val Cys Trp Thr Ser Gly Trp Gly Ala Thr Glu Asp Gly Gly Asp Ala  
 340 345 350

Ser Pro Val Leu Asn His Ala Ala Val Pro Leu Ile Ser Asn Lys Ile  
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Cys Asn His Arg Asp Val Tyr Gly Gly Ile Ile Ser Pro Ser Met Leu  
 370 375 380

Cys Ala Gly Tyr Leu Thr Gly Gly Val Asp Ser Cys Gln Gly Asp Ser  
 385 390 395 400

Gly Gly Pro Leu Val Cys Gln Glu Arg Arg Leu Trp Lys Leu Val Gly  
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Ala Thr Ser Phe Gly Ile Gly Cys Ala Glu Val Asn Lys Pro Gly Val  
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Arg Asp Leu Lys Thr  
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<223> OTHER INFORMATION: Oligonucleotide primer

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<210> SEQ ID NO 39  
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agtgtggggt caaatagttt catgttcccc aaatggccca aaactgacag tttaaacgct 420

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&lt;210&gt; SEQ ID NO 46

&lt;211&gt; LENGTH: 3908

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Escherichia coli

&lt;400&gt; SEQUENCE: 46

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tcactcatta ggcacccccg gctttacact ttatgcttcc ggctcgtatg ttgtgtggaa	180
ttgtgagcgg ataacaattt cacacaggaa acagctatga ccatgattac gccaaagcttg	240
gtaccgagct cggatccact agtaaccggc gccagtgtgc tggaaattcg ccttaagggc	300
gaattctgca gatattccat aactggcgg ccgctcgagc atgcatctag agggccaat	360
tcgccctata gtgagtcgta ttacaattca ctggccgctg ttttacaacg tcgtgactgg	420
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gtgaccgcta cacttgccag cgccctagcg cccgctcctt tcgctttctt cccttccttt	660
ctcgccacgt tcgcccgtt tcccctcaa gctctaaatc gggggctccc tttagggttc	720
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&lt;210&gt; SEQ ID NO 47

&lt;211&gt; LENGTH: 46

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Oligonucleotide primer

&lt;400&gt; SEQUENCE: 47

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&lt;210&gt; SEQ ID NO 48

&lt;211&gt; LENGTH: 32

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Oligonucleotide primer

&lt;400&gt; SEQUENCE: 48

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&lt;210&gt; SEQ ID NO 49

&lt;211&gt; LENGTH: 3147

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo Sapien

&lt;220&gt; FEATURE:

&lt;221&gt; NAME/KEY: CDS

&lt;222&gt; LOCATION: (1865)...(2590)

&lt;223&gt; OTHER INFORMATION: Nucleic acid sequence of protease domain of MTSP1

&lt;400&gt; SEQUENCE: 49

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Val Ser Leu His Ala Leu Gly Gln Gly His Ile Cys Gly Ala Ser Leu	
20 25 30	
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Ile Ser Pro Asn Trp Leu Val Ser Ala Ala His Cys Tyr Ile Asp Asp	
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ctc	aag	cgc	atc	atc	tcc	cac	ccc	ttc	ttc	aat	gac	ttc	acc	ttc	gac	2149
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tgc	gtg	ggc	ttc	ctc	agc	ggc	ggc	gtg	gac	tcc	tgc	cag	ggt	gat	tcc	2437
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	240															
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<210> SEQ ID NO 50  
 <211> LENGTH: 241  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo Sapien  
 <400> SEQUENCE: 50

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 Ser Pro Asn Trp Leu Val Ser Ala Ala His Cys Tyr Ile Asp Asp Arg  
 35 40 45  
 Gly Phe Arg Tyr Ser Asp Pro Thr Gln Trp Thr Ala Phe Leu Gly Leu  
 50 55 60  
 His Asp Gln Ser Gln Arg Ser Ala Pro Gly Val Gln Glu Arg Arg Leu  
 65 70 75 80  
 Lys Arg Ile Ile Ser His Pro Phe Phe Asn Asp Phe Thr Phe Asp Tyr  
 85 90 95  
 Asp Ile Ala Leu Leu Glu Leu Glu Lys Pro Ala Glu Tyr Ser Ser Met  
 100 105 110  
 Val Arg Pro Ile Cys Leu Pro Asp Ala Ser His Val Phe Pro Ala Gly  
 115 120 125  
 Lys Ala Ile Trp Val Thr Gly Trp Gly His Thr Gln Tyr Gly Gly Thr  
 130 135 140  
 Gly Ala Leu Ile Leu Gln Lys Gly Glu Ile Arg Val Ile Asn Gln Thr  
 145 150 155 160  
 Thr Cys Glu Asn Leu Leu Pro Gln Gln Ile Thr Pro Arg Met Met Cys  
 165 170 175  
 Val Gly Phe Leu Ser Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly  
 180 185 190  
 Gly Pro Leu Ser Ser Val Glu Ala Asp Gly Arg Ile Phe Gln Ala Gly  
 195 200 205  
 Val Val Ser Trp Gly Asp Gly Cys Ala Gln Arg Asn Lys Pro Gly Val  
 210 215 220  
 Tyr Thr Arg Leu Pro Leu Phe Arg Asp Trp Ile Lys Glu Asn Thr Gly  
 225 230 235 240  
 Val

<210> SEQ ID NO 51  
 <211> LENGTH: 46  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 51

tctctcgaga aaagagtggg ggggggggtgg ggaggaggcc tctgtg 46

<210> SEQ ID NO 52  
 <211> LENGTH: 43  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial sequence

<400> SEQUENCE: 52

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<210> SEQ ID NO 53  
 <211> LENGTH: 34  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:

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<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 53

attcgcggcc gcattacagc tcagccttcc agac 34

<210> SEQ ID NO 54  
 <211> LENGTH: 43  
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 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 54

ggcacagtca ggcccatctc tctgcccttc tttgatgagg agc 43

<210> SEQ ID NO 55  
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 <212> TYPE: DNA  
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<400> SEQUENCE: 55

cacccttct tcaatgactt caccttgc 28

<210> SEQ ID NO 56  
 <211> LENGTH: 18  
 <212> TYPE: DNA  
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 <220> FEATURE:  
 <223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 56

tacctctcct acgactcc 18

<210> SEQ ID NO 57  
 <211> LENGTH: 25  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 57

gaggttctcg caggtgtctt ggttg 25

<210> SEQ ID NO 58  
 <211> LENGTH: 39  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 58

ctcgagaaaa gagttgttgg gggcacggat gcggatgag 39

<210> SEQ ID NO 59  
 <211> LENGTH: 11  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo Sapien

<400> SEQUENCE: 59

Phe Glu Val Phe Ser Gln Ser Ser Ser Leu Gly  
 1 5 10

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<210> SEQ ID NO 60  
 <211> LENGTH: 32  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo Sapien

<400> SEQUENCE: 60

Glu Ile Val Ala Pro Arg Glu Arg Ala Asp Arg Arg Gly Arg Lys Leu  
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 Leu Cys Trp Arg Lys Pro Thr Lys Met Lys Gly Pro Arg Pro Ser His  
 20 25 30

<210> SEQ ID NO 61  
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 <212> TYPE: DNA  
 <213> ORGANISM: Homo Sapien  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
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 <223> OTHER INFORMATION: Nucleotide sequence encoding corin  
 <308> DATABASE ACCESSION NUMBER: GenBank AF133845  
 <309> DATABASE ENTRY DATE: 1999-05-24

<400> SEQUENCE: 61

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 Met Lys Gln Ser Pro Ala Leu  
 1 5  
 gct ccg gaa gag cgc tac cgc aga gcc ggg tcc cca aag ccg gtc ttg 162  
 Ala Pro Glu Glu Arg Tyr Arg Arg Ala Gly Ser Pro Lys Pro Val Leu  
 10 15 20  
 aga gct gat gac aat aac atg ggc aat ggc tgc tct cag aag ctg gcg 210  
 Arg Ala Asp Asp Asn Asn Met Gly Asn Gly Cys Ser Gln Lys Leu Ala  
 25 30 35  
 act gct aac ctc ctc cgg ttc cta ttg ctg gtc ctg att cca tgt atc 258  
 Thr Ala Asn Leu Leu Arg Phe Leu Leu Leu Val Leu Ile Pro Cys Ile  
 40 45 50 55  
 tgt gct ctc gtt ctc ttg ctg gtg atc ctg ctt tcc tat gtt gga aca 306  
 Cys Ala Leu Val Leu Leu Leu Val Ile Leu Leu Ser Tyr Val Gly Thr  
 60 65 70  
 tta caa aag gtc tat ttt aaa tca aat ggg agt gaa cct ttg gtc act 354  
 Leu Gln Lys Val Tyr Phe Lys Ser Asn Gly Ser Glu Pro Leu Val Thr  
 75 80 85  
 gat ggt gaa atc caa ggg tcc gat gtt att ctt aca aat aca att tat 402  
 Asp Gly Glu Ile Gln Gly Ser Asp Val Ile Leu Thr Asn Thr Ile Tyr  
 90 95 100  
 aac cag agc act gtg gtg tct act gca cat ccc gac caa cac gtt cca 450  
 Asn Gln Ser Thr Val Val Ser Thr Ala His Pro Asp Gln His Val Pro  
 105 110 115  
 gcc tgg act acg gat gct tct ctc cca ggg gac caa agt cac agg aat 498  
 Ala Trp Thr Thr Asp Ala Ser Leu Pro Gly Asp Gln Ser His Arg Asn  
 120 125 130 135  
 aca agt gcc tgt atg aac atc acc cac agc cag tgt cag atg ctg ccc 546  
 Thr Ser Ala Cys Met Asn Ile Thr His Ser Gln Cys Gln Met Leu Pro  
 140 145 150  
 tac cac gcc acg ctg aca cct ctc ctc tca gtt gtc aga aac atg gaa 594  
 Tyr His Ala Thr Leu Thr Pro Leu Leu Ser Val Val Arg Asn Met Glu  
 155 160 165  
 atg gaa aag ttc ctc aag ttt ttc aca tat ctc cat cgc ctc agt tgc 642



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Ser	Tyr	Pro	Asn	Tyr	Phe	Gly	His	Arg	Thr	Gln	Lys	Glu	Ala	Ser	Ile		
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Ser	Trp	Glu	Ser	Ser	Leu	Phe	Ser	Pro	Ala	Leu	Val	Gln	Thr	Asn	Cys	Tyr	
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aaa	tac	ctc	atg	ttc	ttt	tct	tgc	acc	att	ttg	gta	cca	aaa	tgt	gat	1650	
Lys	Tyr	Leu	Met	Phe	Phe	Ser	Cys	Thr	Ile	Leu	Val	Pro	Lys	Cys	Asp		
	505				510					515							
gtg	aat	aca	ggc	gag	cgt	atc	cct	cct	tgc	agg	gca	ttg	tgt	gaa	cac	1698	
Val	Asn	Thr	Gly	Glu	Arg	Ile	Pro	Pro	Cys	Arg	Ala	Leu	Cys	Glu	His		
520				525					530				535				
tct	aaa	gaa	cgc	tgt	gag	tct	gtt	ctt	ggg	att	gtg	ggc	cta	cag	tgg	1746	
Ser	Lys	Glu	Arg	Cys	Glu	Ser	Val	Leu	Gly	Ile	Val	Gly	Leu	Gln	Trp		
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cct	gaa	gac	aca	gat	tgc	agt	caa	ttt	cca	gag	gaa	aat	tca	gac	aat	1794	
Pro	Glu	Asp	Thr	Asp	Cys	Ser	Gln	Phe	Pro	Glu	Glu	Asn	Ser	Asp	Asn		
			555				560					565					
caa	acc	tgc	ctg	atg	cct	gat	gaa	tat	gtg	gaa	gaa	tgc	tca	cct	agt	1842	
Gln	Thr	Cys	Leu	Met	Pro	Asp	Glu	Tyr	Val	Glu	Glu	Cys	Ser	Pro	Ser		
	570					575						580					
cat	ttc	aag	tgc	cgc	tca	gga	cag	tgt	gtt	ctg	gct	tcc	aga	aga	tgt	1890	
His	Phe	Lys	Cys	Arg	Ser	Gly	Gln	Cys	Val	Leu	Ala	Ser	Arg	Arg	Cys		
	585				590					595							
gat	ggc	cag	gcc	gac	tgt	gac	gat	gac	agt	gat	gag	gaa	aac	tgt	ggc	1938	
Asp	Gly	Gln	Ala	Asp	Cys	Asp	Asp	Asp	Ser	Asp	Glu	Glu	Asn	Cys	Gly		
600				605					610					615			
tgt	aaa	gag	aga	gat	ctt	tgg	gaa	tgt	cca	tcc	aat	aaa	caa	tgt	ttg	1986	
Cys	Lys	Glu	Arg	Asp	Leu	Trp	Glu	Cys	Pro	Ser	Asn	Lys	Gln	Cys	Leu		
			620						625				630				
aag	cac	aca	gtg	atc	tgc	gat	ggg	ttc	cca	gac	tgc	cct	gat	tac	atg	2034	
Lys	His	Thr	Val	Ile	Cys	Asp	Gly	Phe	Pro	Asp	Cys	Pro	Asp	Tyr	Met		
			635				640						645				
gac	gag	aaa	aac	tgc	tca	ttt	tgc	caa	gat	gat	gag	ctg	gaa	tgt	gca	2082	
Asp	Glu	Lys	Asn	Cys	Ser	Phe	Cys	Gln	Asp	Asp	Glu	Leu	Glu	Cys	Ala		
		650				655						660					
aac	cat	gcg	tgt	gtg	tca	cgt	gac	ctg	tgg	tgt	gat	ggc	gaa	gcc	gac	2130	
Asn	His	Ala	Cys	Val	Ser	Arg	Asp	Leu	Trp	Cys	Asp	Gly	Glu	Ala	Asp		
	665				670					675							
tgc	tca	gac	agt	tca	gat	gaa	tgg	gac	tgt	gtg	acc	ctc	tct	ata	aat	2178	
Cys	Ser	Asp	Ser	Ser	Asp	Glu	Trp	Asp	Cys	Val	Thr	Leu	Ser	Ile	Asn		
680					685					690				695			
gtg	aac	tcc	tct	tcc	ttt	ctg	atg	gtt	cac	aga	gct	gcc	aca	gaa	cac	2226	
Val	Asn	Ser	Ser	Ser	Phe	Leu	Met	Val	His	Arg	Ala	Ala	Thr	Glu	His		
				700					705				710				
cat	gtg	tgt	gca	gat	ggc	tgg	cag	gag	ata	ttg	agt	cag	ctg	gcc	tgc	2274	
His	Val	Cys	Ala	Asp	Gly	Trp	Gln	Glu	Ile	Leu	Ser	Gln	Leu	Ala	Cys		
			715				720						725				
aag	cag	atg	ggc	tta	gga	gaa	cca	tct	gtg	acc	aaa	ttg	ata	cag	gaa	2322	
Lys	Gln	Met	Gly	Leu	Gly	Glu	Pro	Ser	Val	Thr	Lys	Leu	Ile	Gln	Glu		
		730					735					740					
cag	gag	aaa	gag	ccg	cgg	tgg	ctg	aca	tta	cac	tcc	aac	tgg	gag	agc	2370	
Gln	Glu	Lys	Glu	Pro	Arg	Trp	Leu	Thr	Leu	His	Ser	Asn	Trp	Glu	Ser		
		745				750						755					
ctc	aat	ggg	acc	act	tta	cat	gaa	ctt	cta	gta	aat	ggg	cag	tct	tgt	2418	
Leu	Asn	Gly	Thr	Thr	Leu	His	Glu	Leu	Leu	Val	Asn	Gly	Gln	Ser	Cys		
760				765					770				775				
gag	agc	aga	agt	aaa	att	tct	ctt	ctg	tgt	act	aaa	caa	gac	tgt	ggg	2466	

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Glu Ser Arg Ser Lys Ile Ser Leu Leu Cys Thr Lys Gln Asp Cys Gly	
	780 785 790
cgc cgc cct gct gcc cga atg aac aaa agg atc ctt gga ggt cgg acg	2514
Arg Arg Pro Ala Ala Arg Met Asn Lys Arg Ile Leu Gly Gly Arg Thr	
	795 800 805
agt cgc cct gga agg tgg cca tgg cag tgt tct ctg cag agt gaa ccc	2562
Ser Arg Pro Gly Arg Trp Pro Trp Gln Cys Ser Leu Gln Ser Glu Pro	
	810 815 820
agt gga cat atc tgt gcc tgt gtc ctc att gcc aag aag tgg gtt ctg	2610
Ser Gly His Ile Cys Gly Cys Val Leu Ile Ala Lys Lys Trp Val Leu	
	825 830 835
aca gtt gcc cac tgc ttc gag ggg aga gag aat gct gca gtt tgg aaa	2658
Thr Val Ala His Cys Phe Glu Gly Arg Glu Asn Ala Ala Val Trp Lys	
	840 845 850 855
gtg gtg ctt gcc atc aac aat cta gac cat cca tca gtg ttc atg cag	2706
Val Val Leu Gly Ile Asn Asn Leu Asp His Pro Ser Val Phe Met Gln	
	860 865 870
aca cgc ttt gtg aag acc atc atc ctg cat ccc cgc tac agt cga gca	2754
Thr Arg Phe Val Lys Thr Ile Ile Leu His Pro Arg Tyr Ser Arg Ala	
	875 880 885
gtg gtg gac tat gac atc agc atc gtt gag ctg agt gaa gac atc agt	2802
Val Val Asp Tyr Asp Ile Ser Ile Val Glu Leu Ser Glu Asp Ile Ser	
	890 895 900
gag act ggc tac gtc cgg cct gtc tgc ttg ccc aac ccg gag cag tgg	2850
Glu Thr Gly Tyr Val Arg Pro Val Cys Leu Pro Asn Pro Glu Gln Trp	
	905 910 915
cta gag cct gac acg tac tgc tat atc aca ggc tgg ggc cac atg ggc	2898
Leu Glu Pro Asp Thr Tyr Cys Tyr Ile Thr Gly Trp Gly His Met Gly	
	920 925 930 935
aat aaa atg cca ttt aag ctg caa gag gga gag gtc cgc att att tct	2946
Asn Lys Met Pro Phe Lys Leu Gln Glu Gly Glu Val Arg Ile Ile Ser	
	940 945 950
ctg gaa cat tgt cag tcc tac ttt gac atg aag acc atc acc act cgg	2994
Leu Glu His Cys Gln Ser Tyr Phe Asp Met Lys Thr Ile Thr Thr Arg	
	955 960 965
atg ata tgt gct gcc tat gag tct gcc aca gtt gat tca tgc atg ggt	3042
Met Ile Cys Ala Gly Tyr Glu Ser Gly Thr Val Asp Ser Cys Met Gly	
	970 975 980
gac agc ggt ggg cct ctt gtt tgt gag aag cct gga gga cgg tgg aca	3090
Asp Ser Gly Gly Pro Leu Val Cys Glu Lys Pro Gly Gly Arg Trp Thr	
	985 990 995
tta ttt gga tta act tca tgg gcc tcc gtc tgc ttt tcc aaa gtc ctg	3138
Leu Phe Gly Leu Thr Ser Trp Gly Ser Val Cys Phe Ser Lys Val Leu	
	1000 1005 1010 1015
ggg cct gcc gtt tat agt aat gtg tca tat ttc gtc gaa tgg att aaa	3186
Gly Pro Gly Val Tyr Ser Asn Val Ser Tyr Phe Val Glu Trp Ile Lys	
	1020 1025 1030
aga cag att tac atc cag acc ttt ctc cta aac taa ttataaggat	3232
Arg Gln Ile Tyr Ile Gln Thr Phe Leu Leu Asn *	
	1035 1040
gatcagagac ttttgcacg tacactaaaa gaaaatggcc ttcttgactg tgaagagctg	3292
cctgcagaga gctgtacaga agcacttttc atggacagaa atgotcaatc gtgcactgca	3352
aatttgcatg tttgttttgg actaattttt ttcaatttat tttttcacct tcatttttot	3412
cttatttcaa gttcaatgaa agactttaca aaagcaaaca aagcagactt tgtccttttg	3472
ccaggcctaa ccatgactgc agcacaaaat tatcgactct ggcgagattt aaaatcaggt	3532

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gctacagtaa caggttatgg aatggtctct tttatcctat cacaaaaaa gacatagata 3592
tttaggctga ttaattatct ctaccagttt ttgtttctca agctcagtgc atagtggtaa 3652
atttcagtggt taacattgga gacttgcttt tctttttctt tttttatacc ccacaattot 3712
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tatacatgtg ccatggtggg gtgctgaacc cagtaactcg tcatttgatt tattaanaagc 3832
caagataatt tacatgttta aagtatttac tattaccccc ttctaagtgt tgcataattc 3892
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tgggtttggt caggatatat gagggtgctg attgagactg caacaactac atctatattt 4132
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<210> SEQ ID NO 62
<211> LENGTH: 1042
<212> TYPE: PRT
<213> ORGANISM: Homo Sapien
    
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<400> SEQUENCE: 62

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Gly Ser Pro Lys Pro Val Leu Arg Ala Asp Asp Asn Asn Met Gly Asn
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Gly Cys Ser Gln Lys Leu Ala Thr Ala Asn Leu Leu Arg Phe Leu Leu
          35             40             45
Leu Val Leu Ile Pro Cys Ile Cys Ala Leu Val Leu Leu Leu Val Ile
          50             55             60
Leu Leu Ser Tyr Val Gly Thr Leu Gln Lys Val Tyr Phe Lys Ser Asn
65             70             75             80
Gly Ser Glu Pro Leu Val Thr Asp Gly Glu Ile Gln Gly Ser Asp Val
          85             90             95
Ile Leu Thr Asn Thr Ile Tyr Asn Gln Ser Thr Val Val Ser Thr Ala
    
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100					105					110					
His	Pro	Asp	Gln	His	Val	Pro	Ala	Trp	Thr	Thr	Asp	Ala	Ser	Leu	Pro
		115					120					125			
Gly	Asp	Gln	Ser	His	Arg	Asn	Thr	Ser	Ala	Cys	Met	Asn	Ile	Thr	His
	130					135					140				
Ser	Gln	Cys	Gln	Met	Leu	Pro	Tyr	His	Ala	Thr	Leu	Thr	Pro	Leu	Leu
145					150					155					160
Ser	Val	Val	Arg	Asn	Met	Glu	Met	Glu	Lys	Phe	Leu	Lys	Phe	Phe	Thr
				165					170						175
Tyr	Leu	His	Arg	Leu	Ser	Cys	Tyr	Gln	His	Ile	Met	Leu	Phe	Gly	Cys
		180						185						190	
Thr	Leu	Ala	Phe	Pro	Glu	Cys	Ile	Ile	Asp	Gly	Asp	Asp	Ser	His	Gly
		195					200					205			
Leu	Leu	Pro	Cys	Arg	Ser	Phe	Cys	Glu	Ala	Ala	Lys	Glu	Gly	Cys	Glu
	210					215					220				
Ser	Val	Leu	Gly	Met	Val	Asn	Tyr	Ser	Trp	Pro	Asp	Phe	Leu	Arg	Cys
225					230					235					240
Ser	Gln	Phe	Arg	Asn	Gln	Thr	Glu	Ser	Ser	Asn	Val	Ser	Arg	Ile	Cys
				245					250					255	
Phe	Ser	Pro	Gln	Gln	Glu	Asn	Gly	Lys	Gln	Leu	Leu	Cys	Gly	Arg	Gly
			260					265						270	
Glu	Asn	Phe	Leu	Cys	Ala	Ser	Gly	Ile	Cys	Ile	Pro	Gly	Lys	Leu	Gln
		275					280					285			
Cys	Asn	Gly	Tyr	Asn	Asp	Cys	Asp	Asp	Trp	Ser	Asp	Glu	Ala	His	Cys
	290					295					300				
Asn	Cys	Ser	Glu	Asn	Leu	Phe	His	Cys	His	Thr	Gly	Lys	Cys	Leu	Asn
305				310						315					320
Tyr	Ser	Leu	Val	Cys	Asp	Gly	Tyr	Asp	Asp	Cys	Gly	Asp	Leu	Ser	Asp
				325					330					335	
Glu	Gln	Asn	Cys	Asp	Cys	Asn	Pro	Thr	Thr	Glu	His	Arg	Cys	Gly	Asp
		340						345						350	
Gly	Arg	Cys	Ile	Ala	Met	Glu	Trp	Val	Cys	Asp	Gly	Asp	His	Asp	Cys
		355					360					365			
Val	Asp	Lys	Ser	Asp	Glu	Val	Asn	Cys	Ser	Cys	His	Ser	Gln	Gly	Leu
	370					375					380				
Val	Glu	Cys	Arg	Asn	Gly	Gln	Cys	Ile	Pro	Ser	Thr	Phe	Gln	Cys	Asp
385				390						395					400
Gly	Asp	Glu	Asp	Cys	Lys	Asp	Gly	Ser	Asp	Glu	Glu	Asn	Cys	Ser	Val
				405					410					415	
Ile	Gln	Thr	Ser	Cys	Gln	Glu	Gly	Asp	Gln	Arg	Cys	Leu	Tyr	Asn	Pro
			420					425						430	
Cys	Leu	Asp	Ser	Cys	Gly	Gly	Ser	Ser	Leu	Cys	Asp	Pro	Asn	Asn	Ser
		435					440					445			
Leu	Asn	Asn	Cys	Ser	Gln	Cys	Glu	Pro	Ile	Thr	Leu	Glu	Leu	Cys	Met
	450					455					460				
Asn	Leu	Pro	Tyr	Asn	Ser	Thr	Ser	Tyr	Pro	Asn	Tyr	Phe	Gly	His	Arg
465				470						475					480
Thr	Gln	Lys	Glu	Ala	Ser	Ile	Ser	Trp	Glu	Ser	Ser	Leu	Phe	Pro	Ala
				485					490					495	
Leu	Val	Gln	Thr	Asn	Cys	Tyr	Lys	Tyr	Leu	Met	Phe	Phe	Ser	Cys	Thr
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Ile Leu Val Pro Lys Cys Asp Val Asn Thr Gly Glu Arg Ile Pro Pro  
515 520 525

Cys Arg Ala Leu Cys Glu His Ser Lys Glu Arg Cys Glu Ser Val Leu  
530 535 540

Gly Ile Val Gly Leu Gln Trp Pro Glu Asp Thr Asp Cys Ser Gln Phe  
545 550 555 560

Pro Glu Glu Asn Ser Asp Asn Gln Thr Cys Leu Met Pro Asp Glu Tyr  
565 570 575

Val Glu Glu Cys Ser Pro Ser His Phe Lys Cys Arg Ser Gly Gln Cys  
580 585 590

Val Leu Ala Ser Arg Arg Cys Asp Gly Gln Ala Asp Cys Asp Asp Asp  
595 600 605

Ser Asp Glu Glu Asn Cys Gly Cys Lys Glu Arg Asp Leu Trp Glu Cys  
610 615 620

Pro Ser Asn Lys Gln Cys Leu Lys His Thr Val Ile Cys Asp Gly Phe  
625 630 635 640

Pro Asp Cys Pro Asp Tyr Met Asp Glu Lys Asn Cys Ser Phe Cys Gln  
645 650 655

Asp Asp Glu Leu Glu Cys Ala Asn His Ala Cys Val Ser Arg Asp Leu  
660 665 670

Trp Cys Asp Gly Glu Ala Asp Cys Ser Asp Ser Ser Asp Glu Trp Asp  
675 680 685

Cys Val Thr Leu Ser Ile Asn Val Asn Ser Ser Ser Phe Leu Met Val  
690 695 700

His Arg Ala Ala Thr Glu His His Val Cys Ala Asp Gly Trp Gln Glu  
705 710 715 720

Ile Leu Ser Gln Leu Ala Cys Lys Gln Met Gly Leu Gly Glu Pro Ser  
725 730 735

Val Thr Lys Leu Ile Gln Glu Gln Glu Lys Glu Pro Arg Trp Leu Thr  
740 745 750

Leu His Ser Asn Trp Glu Ser Leu Asn Gly Thr Thr Leu His Glu Leu  
755 760 765

Leu Val Asn Gly Gln Ser Cys Glu Ser Arg Ser Lys Ile Ser Leu Leu  
770 775 780

Cys Thr Lys Gln Asp Cys Gly Arg Arg Pro Ala Ala Arg Met Asn Lys  
785 790 795 800

Arg Ile Leu Gly Gly Arg Thr Ser Arg Pro Gly Arg Trp Pro Trp Gln  
805 810 815

Cys Ser Leu Gln Ser Glu Pro Ser Gly His Ile Cys Gly Cys Val Leu  
820 825 830

Ile Ala Lys Lys Trp Val Leu Thr Val Ala His Cys Phe Glu Gly Arg  
835 840 845

Glu Asn Ala Ala Val Trp Lys Val Val Leu Gly Ile Asn Asn Leu Asp  
850 855 860

His Pro Ser Val Phe Met Gln Thr Arg Phe Val Lys Thr Ile Ile Leu  
865 870 875 880

His Pro Arg Tyr Ser Arg Ala Val Val Asp Tyr Asp Ile Ser Ile Val  
885 890 895

Glu Leu Ser Glu Asp Ile Ser Glu Thr Gly Tyr Val Arg Pro Val Cys  
900 905 910

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Leu Pro Asn Pro Glu Gln Trp Leu Glu Pro Asp Thr Tyr Cys Tyr Ile  
 915 920 925  
 Thr Gly Trp Gly His Met Gly Asn Lys Met Pro Phe Lys Leu Gln Glu  
 930 935 940  
 Gly Glu Val Arg Ile Ile Ser Leu Glu His Cys Gln Ser Tyr Phe Asp  
 945 950 955 960  
 Met Lys Thr Ile Thr Thr Arg Met Ile Cys Ala Gly Tyr Glu Ser Gly  
 965 970 975  
 Thr Val Asp Ser Cys Met Gly Asp Ser Gly Gly Pro Leu Val Cys Glu  
 980 985 990  
 Lys Pro Gly Gly Arg Trp Thr Leu Phe Gly Leu Thr Ser Trp Gly Ser  
 995 1000 1005  
 Val Cys Phe Ser Lys Val Leu Gly Pro Gly Val Tyr Ser Asn Val Ser  
 1010 1015 1020  
 Tyr Phe Val Glu Trp Ile Lys Arg Gln Ile Tyr Ile Gln Thr Phe Leu  
 1025 1030 1035 1040

Leu Asn

<210> SEQ ID NO 63  
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 <220> FEATURE:  
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 <309> DATABASE ENTRY DATE: 1995-06-03

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 ggc ata tct tct agg cat cat tct ctc agc tcc tat gaa atc atg ttt 103  
 Gly Ile Ser Ser Arg His His Ser Leu Ser Ser Tyr Glu Ile Met Phe  
 10 15 20  
 gca gct ctc ttt gcc ata ttg gta gtg ctc tgt gct gga tta att gca 151  
 Ala Ala Leu Phe Ala Ile Leu Val Val Leu Cys Ala Gly Leu Ile Ala  
 25 30 35  
 gta tcc tgc ctg aca atc aag gaa tcc caa cga ggt gca gca ctt gga 199  
 Val Ser Cys Leu Thr Ile Lys Glu Ser Gln Arg Gly Ala Ala Leu Gly  
 40 45 50  
 cag agt cat gaa gcc aga gcg aca ttt aaa ata aca tcc gga gtt aca 247  
 Gln Ser His Glu Ala Arg Ala Thr Phe Lys Ile Thr Ser Gly Val Thr  
 55 60 65  
 tat aat cct aat ttg caa gac aaa ctc tca gtg gat ttc aaa gtt ctt 295  
 Tyr Asn Pro Asn Leu Gln Asp Lys Leu Ser Val Asp Phe Lys Val Leu  
 70 75 80 85  
 gct ttt gac ctt cag caa atg ata gat gag atc ttt cta tca agc aat 343  
 Ala Phe Asp Leu Gln Gln Met Ile Asp Glu Ile Phe Leu Ser Ser Asn  
 90 95 100  
 ctg aag aat gaa tat aag aac tca aga gtt tta caa ttt gaa aat ggc 391  
 Leu Lys Asn Glu Tyr Lys Asn Ser Arg Val Leu Gln Phe Glu Asn Gly  
 105 110 115  
 agc att ata gtc gta ttt gac ctt ttc ttt gcc cag tgg gtg tca gat 439  
 Ser Ile Ile Val Val Phe Asp Leu Phe Phe Ala Gln Trp Val Ser Asp  
 120 125 130

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caa aat gta aaa gaa gaa ctg att caa ggc ctt gaa gca aat aaa tcc	487
Gln Asn Val Lys Glu Glu Leu Ile Gln Gly Leu Glu Ala Asn Lys Ser	
135 140 145	
agc caa ctg gtc act ttc cat att gat ttg aac agc gtt gat atc cta	535
Ser Gln Leu Val Thr Phe His Ile Asp Leu Asn Ser Val Asp Ile Leu	
150 155 160 165	
gac aag cta aca acc acc agt cat ctg gca act cca gga aat gtc tca	583
Asp Lys Leu Thr Thr Thr Ser His Leu Ala Thr Pro Gly Asn Val Ser	
170 175 180	
ata gag tgc ctg cct ggt tca agt cct tgt act gat gct cta acg tgt	631
Ile Glu Cys Leu Pro Gly Ser Ser Pro Cys Thr Asp Ala Leu Thr Cys	
185 190 195	
ata aaa gct gat tta ttt tgt gat gga gaa gta aac tgt cca gat ggt	679
Ile Lys Ala Asp Leu Phe Cys Asp Gly Glu Val Asn Cys Pro Asp Gly	
200 205 210	
tct gac gaa gac aat aaa atg tgt gcc aca gtt tgt gat gga aga ttt	727
Ser Asp Glu Asp Asn Lys Met Cys Ala Thr Val Cys Asp Gly Arg Phe	
215 220 225	
ttg tta act gga tca tct ggg tct ttc cag gct act cat tat cca aaa	775
Leu Leu Thr Gly Ser Ser Gly Ser Phe Gln Ala Thr His Tyr Pro Lys	
230 235 240 245	
cct tct gaa aca agt gtt gtc tgc cag tgg atc ata cgt gta aac caa	823
Pro Ser Glu Thr Ser Val Val Cys Gln Trp Ile Ile Arg Val Asn Gln	
250 255 260	
gga ctt tcc att aaa ctg agc ttc gat gat ttt aat aca tat tat aca	871
Gly Leu Ser Ile Lys Leu Ser Phe Asp Asp Phe Asn Thr Tyr Tyr Thr	
265 270 275	
gat ata tta gat att tat gaa ggt gta gga tca agc aag att tta aga	919
Asp Ile Leu Asp Ile Tyr Glu Gly Val Gly Ser Ser Lys Ile Leu Arg	
280 285 290	
gct tct att tgg gaa act aat cct ggc aca ata aga att ttt tcc aac	967
Ala Ser Ile Trp Glu Thr Asn Pro Gly Thr Ile Arg Ile Phe Ser Asn	
295 300 305	
caa gtt act gcc acc ttt ctt ata gaa tct gat gaa agt gat tat gtt	1015
Gln Val Thr Ala Thr Phe Leu Ile Glu Ser Asp Glu Ser Asp Tyr Val	
310 315 320 325	
ggc ttt aat gca aca tat act gca ttt aac agc agt gag ctt aat aat	1063
Gly Phe Asn Ala Thr Tyr Thr Ala Phe Asn Ser Ser Glu Leu Asn Asn	
330 335 340	
tat gag aaa att aat tgt aac ttt gag gat ggc ttt tgt ttc tgg gtc	1111
Tyr Glu Lys Ile Asn Cys Asn Phe Glu Asp Gly Phe Cys Phe Trp Val	
345 350 355	
cag gat cta aat gat gat aat gaa tgg gaa agg att cag gga agc acc	1159
Gln Asp Leu Asn Asp Asp Asn Glu Trp Glu Arg Ile Gln Gly Ser Thr	
360 365 370	
ttt tct cct ttt act gga ccc aat ttt gac cac act ttt ggc aat gct	1207
Phe Ser Pro Phe Thr Gly Pro Asn Phe Asp His Thr Phe Gly Asn Ala	
375 380 385	
tca gga ttt tac att tct acc cca act gga cca gga ggg aga caa gaa	1255
Ser Gly Phe Tyr Ile Ser Thr Pro Thr Gly Pro Gly Gly Arg Gln Glu	
390 395 400 405	
cga gtg ggg ctt tta agc ctc cct ttg gac ccc act ttg gag cca gct	1303
Arg Val Gly Leu Leu Ser Leu Pro Leu Asp Pro Thr Leu Glu Pro Ala	
410 415 420	
tgc ctt agt ttc tgg tat cat atg tat ggt gaa aat gtc cat aaa tta	1351
Cys Leu Ser Phe Trp Tyr His Met Tyr Gly Glu Asn Val His Lys Leu	
425 430 435	

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agc att aat atc agc aat gac caa aat atg gag aag aca gtt ttc caa Ser Ile Asn Ile Ser Asn Asp Gln Asn Met Glu Lys Thr Val Phe Gln 440 445 450	1399
aag gaa gga aat tat gga gac aat tgg aat tat gga caa gta acc cta Lys Glu Gly Asn Tyr Gly Asp Asn Trp Asn Tyr Gly Gln Val Thr Leu 455 460 465	1447
aat gaa aca gtt aaa ttt aag gtt gct ttt aat gct ttt aaa aac aag Asn Glu Thr Val Lys Phe Lys Val Ala Phe Asn Ala Phe Lys Asn Lys 470 475 480 485	1495
atc ctg agt gat att gcg ttg gat gac att agc cta aca tat ggg att Ile Leu Ser Asp Ile Ala Leu Asp Asp Ile Ser Leu Thr Tyr Gly Ile 490 495 500	1543
tgc aat ggg agt ctt tat cca gaa cca act ttg gtg cca act cct cca Cys Asn Gly Ser Leu Tyr Pro Glu Pro Thr Leu Val Pro Thr Pro Pro 505 510 515	1591
cca gaa ctt cct acg gac tgt gga gga cct ttt gag ctg tgg gag cca Pro Glu Leu Pro Thr Asp Cys Gly Gly Pro Phe Glu Leu Trp Glu Pro 520 525 530	1639
aat aca aca ttc agt tct acg aac ttt cca aac agc tac cct aat ctg Asn Thr Thr Phe Ser Ser Thr Asn Phe Pro Asn Ser Tyr Pro Asn Leu 535 540 545	1687
gct ttc tgt gtt tgg att tta aat gca caa aaa gga aag aat ata caa Ala Phe Cys Val Trp Ile Leu Asn Ala Gln Lys Gly Lys Asn Ile Gln 550 555 560 565	1735
ctt cat ttt caa gaa ttt gac tta gaa aat att aac gat gta gtt gaa Leu His Phe Gln Glu Phe Asp Leu Glu Asn Ile Asn Asp Val Val Glu 570 575 580	1783
ata aga gat ggt gaa gaa gct gat tcc ttg ctc tta gct gtg tac aca Ile Arg Asp Gly Glu Glu Ala Asp Ser Leu Leu Leu Ala Val Tyr Thr 585 590 595	1831
ggg cct ggc cca gta aag gat gtg ttc tct acc acc aac aga atg act Gly Pro Gly Pro Val Lys Asp Val Phe Ser Thr Thr Asn Arg Met Thr 600 605 610	1879
gtg ctt ctc atc act aac gat gtg ttg gca aga gga ggg ttt aaa gca Val Leu Leu Ile Thr Asn Asp Val Leu Ala Arg Gly Gly Phe Lys Ala 615 620 625	1927
aac ttt act act ggc tat cac ttg ggg att cca gag cca tgc aag gca Asn Phe Thr Thr Gly Tyr His Leu Gly Ile Pro Glu Pro Cys Lys Ala 630 635 640 645	1975
gac cat ttt caa tgt aaa aat gga gag tgt gtt cca ctg gtg aat ctc Asp His Phe Gln Cys Lys Asn Gly Glu Cys Val Pro Leu Val Asn Leu 650 655 660	2023
tgt gac ggt cat ctg cac tgt gag gat ggc tca gat gaa gca gat tgt Cys Asp Gly His Leu His Cys Glu Asp Gly Ser Asp Glu Ala Asp Cys 665 670 675	2071
gtg cgt ttt ttc aat ggc aca acg aac aac aat ggt tta gtg cgg ttc Val Arg Phe Phe Asn Gly Thr Asn Asn Asn Gly Leu Val Arg Phe 680 685 690	2119
aga atc cag agc ata tgg cat aca gct tgt gct gag aac tgg acc acc Arg Ile Gln Ser Ile Trp His Thr Ala Cys Ala Glu Asn Trp Thr Thr 695 700 705	2167
cag att tca aat gat gtt tgt caa ctg ctg gga cta ggg agt gga aac Gln Ile Ser Asn Asp Val Cys Gln Leu Leu Gly Leu Gly Ser Gly Asn 710 715 720 725	2215
tca tca aag cca atc ttc tct acc gat ggt gga cca ttt gtc aaa tta Ser Ser Lys Pro Ile Phe Ser Thr Asp Gly Gly Pro Phe Val Lys Leu 730 735 740	2263

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aac aca gca cct gat ggc cac tta ata cta aca ccc agt caa cag tgt	2311
Asn Thr Ala Pro Asp Gly His Leu Ile Leu Thr Pro Ser Gln Gln Cys	
745 750 755	
tta cag gat tcc ttg att cgg tta cag tgt aac cat aaa tct tgt gga	2359
Leu Gln Asp Ser Leu Ile Arg Leu Gln Cys Asn His Lys Ser Cys Gly	
760 765 770	
aaa aaa ctg gca gct caa gac atc acc cca aag att gtt gga gga agt	2407
Lys Lys Leu Ala Ala Gln Asp Ile Thr Pro Lys Ile Val Gly Gly Ser	
775 780 785	
aat gcc aaa gaa ggg gcc tgg ccc tgg gtt gtg ggt ctg tat tat ggc	2455
Asn Ala Lys Glu Gly Ala Trp Pro Trp Val Val Gly Leu Tyr Tyr Gly	
790 795 800 805	
ggc cga ctg ctc tgc ggc gca tct ctc gtc agc agt gac tgg ctg gtg	2503
Gly Arg Leu Leu Cys Gly Ala Ser Leu Val Ser Ser Asp Trp Leu Val	
810 815 820	
tcc gcc gca cac tgc gtg tat ggg aga aac tta gag cca tcc aag tgg	2551
Ser Ala Ala His Cys Val Tyr Gly Arg Asn Leu Glu Pro Ser Lys Trp	
825 830 835	
aca gca atc cta ggc ctg cat atg aaa tca aat ctg acc tct cct caa	2599
Thr Ala Ile Leu Gly Leu His Met Lys Ser Asn Leu Thr Ser Pro Gln	
840 845 850	
aca gtc cct cga tta ata gat gaa att gtc ata aac cct cat tac aat	2647
Thr Val Pro Arg Leu Ile Asp Glu Ile Val Ile Asn Pro His Tyr Asn	
855 860 865	
agg cga aga aag gac aac gac att gcc atg atg cat ctg gaa ttt aaa	2695
Arg Arg Arg Lys Asp Asn Asp Ile Ala Met Met His Leu Glu Phe Lys	
870 875 880 885	
gtg aat tac aca gat tac ata caa cct att tgt tta ccg gaa gaa aat	2743
Val Asn Tyr Thr Asp Tyr Ile Gln Pro Ile Cys Leu Pro Glu Glu Asn	
890 895 900	
caa gtt ttt cct cca gga aga aat tgt tct att gct ggt tgg ggg acg	2791
Gln Val Phe Pro Pro Gly Arg Asn Cys Ser Ile Ala Gly Trp Gly Thr	
905 910 915	
gtt gta tat caa ggt act act gca aac ata ttg caa gaa gct gat gtt	2839
Val Val Tyr Gln Gly Thr Thr Ala Asn Ile Leu Gln Glu Ala Asp Val	
920 925 930	
cct ctt cta tca aat gag aga tgc caa cag cag atg cca gaa tat aac	2887
Pro Leu Leu Ser Asn Glu Arg Cys Gln Gln Gln Met Pro Glu Tyr Asn	
935 940 945	
att act gaa aat atg ata tgt gca ggc tat gaa gaa gga gga ata gat	2935
Ile Thr Glu Asn Met Ile Cys Ala Gly Tyr Glu Glu Gly Gly Ile Asp	
950 955 960 965	
tct tgt cag ggg gat tca gga gga cca tta atg tgc caa gaa aac aac	2983
Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Cys Gln Glu Asn Asn	
970 975 980	
agg tgg ttc ctt gct ggt gtg acc tca ttt gga tac aag tgt gcc ctg	3031
Arg Trp Phe Leu Ala Gly Val Thr Ser Phe Gly Tyr Lys Cys Ala Leu	
985 990 995	
cct aat cgc ccc gga gtg tat gcc agg gtc tca agg ttt acc gaa tgg	3079
Pro Asn Arg Pro Gly Val Tyr Ala Arg Val Ser Arg Phe Thr Glu Trp	
1000 1005 1010	
ata caa agt ttt cta cat tag cgcatttctt aaactaaaca gaaaagtcgc	3130
Ile Gln Ser Phe Leu His *	
1015	
attattttcc cattotactc tagaaagcat ggaaattaag tgtttcgtac aaaaatttta	3190
aaaagttacc aaaggttttt attcttacct atgtcaatga aatgctaggg ggccagggaa	3250

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acaaaatttt aaaaataata aaattcacca tagcaataca gaataacttt aaaataccat 3310
taaatacatt tgtatttcat tgtgaacagg tatttcttca cagatctcat ttttaaatt 3370
cttaatgatt atttttatta cttactgttg tttaaagga tgtattttta aagcatatac 3430
catacactta agaaatttga gcagaattta aaaaagaaag aaaataaatt gtttttccca 3490
aagtatgtca ctgttggaat taaactgcca taaatcttct agttccagtt tagtttgctg 3550
ctattagcag aaactcaatt gtttctctgt cttttctatc aaaattttca acatatgcat 3610
aaccttagta ttttcccaac caatagaaac tatttattgt aagottatgt cacaggcctg 3670
gactaaattg attttacgtt cctctt 3696

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&lt;210&gt; SEQ ID NO 64

&lt;211&gt; LENGTH: 1019

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo Sapien

&lt;400&gt; SEQUENCE: 64

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Tyr Glu Ile Met Phe Ala Ala Leu Phe Ala Ile Leu Val Val Leu Cys
 20           25           30
Ala Gly Leu Ile Ala Val Ser Cys Leu Thr Ile Lys Glu Ser Gln Arg
 35           40           45
Gly Ala Ala Leu Gly Gln Ser His Glu Ala Arg Ala Thr Phe Lys Ile
 50           55           60
Thr Ser Gly Val Thr Tyr Asn Pro Asn Leu Gln Asp Lys Leu Ser Val
 65           70           75           80
Asp Phe Lys Val Leu Ala Phe Asp Leu Gln Gln Met Ile Asp Glu Ile
 85           90           95
Phe Leu Ser Ser Asn Leu Lys Asn Glu Tyr Lys Asn Ser Arg Val Leu
 100          105          110
Gln Phe Glu Asn Gly Ser Ile Ile Val Val Phe Asp Leu Phe Phe Ala
 115          120          125
Gln Trp Val Ser Asp Gln Asn Val Lys Glu Glu Leu Ile Gln Gly Leu
 130          135          140
Glu Ala Asn Lys Ser Ser Gln Leu Val Thr Phe His Ile Asp Leu Asn
 145          150          155          160
Ser Val Asp Ile Leu Asp Lys Leu Thr Thr Thr Ser His Leu Ala Thr
 165          170          175
Pro Gly Asn Val Ser Ile Glu Cys Leu Pro Gly Ser Ser Pro Cys Thr
 180          185          190
Asp Ala Leu Thr Cys Ile Lys Ala Asp Leu Phe Cys Asp Gly Glu Val
 195          200          205
Asn Cys Pro Asp Gly Ser Asp Glu Asp Asn Lys Met Cys Ala Thr Val
 210          215          220
Cys Asp Gly Arg Phe Leu Leu Thr Gly Ser Ser Gly Ser Phe Gln Ala
 225          230          235          240
Thr His Tyr Pro Lys Pro Ser Glu Thr Ser Val Val Cys Gln Trp Ile
 245          250          255
Ile Arg Val Asn Gln Gly Leu Ser Ile Lys Leu Ser Phe Asp Asp Phe
 260          265          270

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Asn Thr Tyr Tyr Thr Asp Ile Leu Asp Ile Tyr Glu Gly Val Gly Ser  
 275 280 285

Ser Lys Ile Leu Arg Ala Ser Ile Trp Glu Thr Asn Pro Gly Thr Ile  
 290 295 300

Arg Ile Phe Ser Asn Gln Val Thr Ala Thr Phe Leu Ile Glu Ser Asp  
 305 310 315 320

Glu Ser Asp Tyr Val Gly Phe Asn Ala Thr Tyr Thr Ala Phe Asn Ser  
 325 330 335

Ser Glu Leu Asn Asn Tyr Glu Lys Ile Asn Cys Asn Phe Glu Asp Gly  
 340 345 350

Phe Cys Phe Trp Val Gln Asp Leu Asn Asp Asp Asn Glu Trp Glu Arg  
 355 360 365

Ile Gln Gly Ser Thr Phe Ser Pro Phe Thr Gly Pro Asn Phe Asp His  
 370 375 380

Thr Phe Gly Asn Ala Ser Gly Phe Tyr Ile Ser Thr Pro Thr Gly Pro  
 385 390 395 400

Gly Gly Arg Gln Glu Arg Val Gly Leu Leu Ser Leu Pro Leu Asp Pro  
 405 410 415

Thr Leu Glu Pro Ala Cys Leu Ser Phe Trp Tyr His Met Tyr Gly Glu  
 420 425 430

Asn Val His Lys Leu Ser Ile Asn Ile Ser Asn Asp Gln Asn Met Glu  
 435 440 445

Lys Thr Val Phe Gln Lys Glu Gly Asn Tyr Gly Asp Asn Trp Asn Tyr  
 450 455 460

Gly Gln Val Thr Leu Asn Glu Thr Val Lys Phe Lys Val Ala Phe Asn  
 465 470 475 480

Ala Phe Lys Asn Lys Ile Leu Ser Asp Ile Ala Leu Asp Asp Ile Ser  
 485 490 495

Leu Thr Tyr Gly Ile Cys Asn Gly Ser Leu Tyr Pro Glu Pro Thr Leu  
 500 505 510

Val Pro Thr Pro Pro Pro Glu Leu Pro Thr Asp Cys Gly Gly Pro Phe  
 515 520 525

Glu Leu Trp Glu Pro Asn Thr Thr Phe Ser Ser Thr Asn Phe Pro Asn  
 530 535 540

Ser Tyr Pro Asn Leu Ala Phe Cys Val Trp Ile Leu Asn Ala Gln Lys  
 545 550 555 560

Gly Lys Asn Ile Gln Leu His Phe Gln Glu Phe Asp Leu Glu Asn Ile  
 565 570 575

Asn Asp Val Val Glu Ile Arg Asp Gly Glu Glu Ala Asp Ser Leu Leu  
 580 585 590

Leu Ala Val Tyr Thr Gly Pro Gly Pro Val Lys Asp Val Phe Ser Thr  
 595 600 605

Thr Asn Arg Met Thr Val Leu Leu Ile Thr Asn Asp Val Leu Ala Arg  
 610 615 620

Gly Gly Phe Lys Ala Asn Phe Thr Thr Gly Tyr His Leu Gly Ile Pro  
 625 630 635 640

Glu Pro Cys Lys Ala Asp His Phe Gln Cys Lys Asn Gly Glu Cys Val  
 645 650 655

Pro Leu Val Asn Leu Cys Asp Gly His Leu His Cys Glu Asp Gly Ser  
 660 665 670

Asp Glu Ala Asp Cys Val Arg Phe Phe Asn Gly Thr Thr Asn Asn Asn



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<308> DATABASE ACCESSION NUMBER: GenBank AB002134  
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 <400> SEQUENCE: 65

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  Met Tyr Arg Pro Ala Arg Val Thr Ser Thr Ser Arg Phe Leu Asn Pro
    1           5           10           15

tat gta gta tgt ttc att gtc gtc gca ggg gta gtg atc ctg gca gtc      157
Tyr Val Val Cys Phe Ile Val Val Ala Gly Val Val Ile Leu Ala Val
    20           25           30

acc ata gct cta ctt gtt tac ttt tta gct ttt gat caa aaa tct tac      205
Thr Ile Ala Leu Leu Val Tyr Phe Leu Ala Phe Asp Gln Lys Ser Tyr
    35           40           45

ttt tat agg agc agt ttt caa ctc cta aat gtt gaa tat aat agt cag      253
Phe Tyr Arg Ser Ser Phe Gln Leu Leu Asn Val Glu Tyr Asn Ser Gln
    50           55           60

tta aat tca cca gct aca cag gaa tac agg act ttg agt gga aga att      301
Leu Asn Ser Pro Ala Thr Gln Glu Tyr Arg Thr Leu Ser Gly Arg Ile
    65           70           75           80

gaa tct ctg att act aaa aca ttc aaa gaa tca aat tta aga aat cag      349
Glu Ser Leu Ile Thr Lys Thr Phe Lys Glu Ser Asn Leu Arg Asn Gln
    85           90           95

ttc atc aga gct cat gtt gcc aaa ctg agg caa gat ggt agt ggt gtg      397
Phe Ile Arg Ala His Val Ala Lys Leu Arg Gln Asp Gly Ser Gly Val
    100          105          110

aga gcg gat gtt gtc atg aaa ttt caa ttc act aga aat aac aat gga      445
Arg Ala Asp Val Val Met Lys Phe Gln Phe Thr Arg Asn Asn Asn Gly
    115          120          125

gca tca atg aaa agc aga att gag tct gtt tta cga caa atg ctg aat      493
Ala Ser Met Lys Ser Arg Ile Glu Ser Val Leu Arg Gln Met Leu Asn
    130          135          140

aac tct gga aac ctg gaa ata aac cct tca act gag ata aca tca ctt      541
Asn Ser Gly Asn Leu Glu Ile Asn Pro Ser Thr Glu Ile Thr Ser Leu
    145          150          155          160

act gac cag gct gca gca aat tgg ctt att aat gaa tgt ggg gcc ggt      589
Thr Asp Gln Ala Ala Ala Asn Trp Leu Ile Asn Glu Cys Gly Ala Gly
    165          170          175

cca gac cta ata aca ttg tct gag cag aga atc ctt gga ggc act gag      637
Pro Asp Leu Ile Thr Leu Ser Glu Gln Arg Ile Leu Gly Gly Thr Glu
    180          185          190

gct gag gag gga agc tgg ccg tgg caa gtc agt ctg cgg ctc aat aat      685
Ala Glu Glu Gly Ser Trp Pro Trp Gln Val Ser Leu Arg Leu Asn Asn
    195          200          205

gcc cac cac tgt gga ggc agc ctg atc aat aac atg tgg atc ctg aca      733
Ala His His Cys Gly Gly Ser Leu Ile Asn Asn Met Trp Ile Leu Thr
    210          215          220

gca gct cac tgc ttc aga agc aac tct aat cct cgt gac tgg att gcc      781
Ala Ala His Cys Phe Arg Ser Asn Ser Asn Pro Arg Asp Trp Ile Ala
    225          230          235          240

acg tct ggt att tcc aca aca ttt cct aaa cta aga atg aga gta aga      829
Thr Ser Gly Ile Ser Thr Thr Phe Pro Lys Leu Arg Met Arg Val Arg
    245          250          255

aat att tta att cat aac aat tat aaa tct gca act cat gaa aat gac      877
Asn Ile Leu Ile His Asn Asn Tyr Lys Ser Ala Thr His Glu Asn Asp
    260          265          270

att gca ctt gtg aga ctt gag aac agt gtc acc ttt acc aaa gat atc      925
    
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His Ser Val Cys Leu Pro Ala Ala Thr Gln Asn Ile Pro Pro Gly Ser
    290                295                300

act gct tat gta aca gga tgg ggc gct caa gaa tat gct ggc cac aca    1021
Thr Ala Tyr Val Thr Gly Trp Gly Ala Gln Glu Tyr Ala Gly His Thr
    305                310                315                320

ggt cca gag cta agg caa gga cag gtc aga ata ata agt aat gat gta    1069
Val Pro Glu Leu Arg Gln Gly Gln Val Arg Ile Ile Ser Asn Asp Val
                325                330                335

tgt aat gca cca cat agt tat aat gga gcc atc ttg tct gga atg ctg    1117
Cys Asn Ala Pro His Ser Tyr Asn Gly Ala Ile Leu Ser Gly Met Leu
                340                345                350

tgt gct gga gta cct caa ggt gga gtg gac gca tgt cag ggt gac tct    1165
Cys Ala Gly Val Pro Gln Gly Gly Val Asp Ala Cys Gln Gly Asp Ser
    355                360                365

ggt ggc cca cta gta caa gaa gac tca cgg cgg ctt tgg ttt att gtg    1213
Gly Gly Pro Leu Val Gln Glu Asp Ser Arg Arg Leu Trp Phe Ile Val
    370                375                380

ggg ata gta agc tgg gga gat cag tgt ggc ctg cgg gat aag cca gga    1261
Gly Ile Val Ser Trp Gly Asp Gln Cys Gly Leu Pro Asp Lys Pro Gly
    385                390                395                400

gtg tat act cga gtg aca gcc tac ctt gac tgg att agg caa caa act    1309
Val Tyr Thr Arg Val Thr Ala Tyr Leu Asp Trp Ile Arg Gln Gln Thr
                405                410                415

ggg atc tag tgcaacaagt gcatcctgt tgcaagtct gtatgcaggt    1358
Gly Ile *

gtgcctgtct taaattccaa agctttacat ttcaactgaa aaagaaacta gaaatgtcct    1418

aatttaacat cttgttacat aaatattggtt taacaaacac tgtttaacct tcttttatta    1478

ttaaagggttt tctattttct cc    1500

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&lt;210&gt; SEQ ID NO 66

&lt;211&gt; LENGTH: 418

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo Sapien

&lt;400&gt; SEQUENCE: 66

```

Met Tyr Arg Pro Ala Arg Val Thr Ser Thr Ser Arg Phe Leu Asn Pro
  1                5                10                15

Tyr Val Val Cys Phe Ile Val Val Ala Gly Val Val Ile Leu Ala Val
    20                25                30

Thr Ile Ala Leu Leu Val Tyr Phe Leu Ala Phe Asp Gln Lys Ser Tyr
    35                40                45

Phe Tyr Arg Ser Ser Phe Gln Leu Leu Asn Val Glu Tyr Asn Ser Gln
    50                55                60

Leu Asn Ser Pro Ala Thr Gln Glu Tyr Arg Thr Leu Ser Gly Arg Ile
    65                70                75                80

Glu Ser Leu Ile Thr Lys Thr Phe Lys Glu Ser Asn Leu Arg Asn Gln
    85                90                95

Phe Ile Arg Ala His Val Ala Lys Leu Arg Gln Asp Gly Ser Gly Val
    100                105                110

Arg Ala Asp Val Val Met Lys Phe Gln Phe Thr Arg Asn Asn Asn Gly
    115                120                125

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Ala Ser Met Lys Ser Arg Ile Glu Ser Val Leu Arg Gln Met Leu Asn
  130                               135                               140

Asn Ser Gly Asn Leu Glu Ile Asn Pro Ser Thr Glu Ile Thr Ser Leu
145                               150                               155                               160

Thr Asp Gln Ala Ala Ala Asn Trp Leu Ile Asn Glu Cys Gly Ala Gly
  165                               170                               175

Pro Asp Leu Ile Thr Leu Ser Glu Gln Arg Ile Leu Gly Gly Thr Glu
  180                               185                               190

Ala Glu Glu Gly Ser Trp Pro Trp Gln Val Ser Leu Arg Leu Asn Asn
  195                               200                               205

Ala His His Cys Gly Gly Ser Leu Ile Asn Asn Met Trp Ile Leu Thr
  210                               215                               220

Ala Ala His Cys Phe Arg Ser Asn Ser Asn Pro Arg Asp Trp Ile Ala
225                               230                               235                               240

Thr Ser Gly Ile Ser Thr Thr Phe Pro Lys Leu Arg Met Arg Val Arg
  245                               250                               255

Asn Ile Leu Ile His Asn Asn Tyr Lys Ser Ala Thr His Glu Asn Asp
  260                               265                               270

Ile Ala Leu Val Arg Leu Glu Asn Ser Val Thr Phe Thr Lys Asp Ile
  275                               280                               285

His Ser Val Cys Leu Pro Ala Ala Thr Gln Asn Ile Pro Pro Gly Ser
  290                               295                               300

Thr Ala Tyr Val Thr Gly Trp Gly Ala Gln Glu Tyr Ala Gly His Thr
305                               310                               315                               320

Val Pro Glu Leu Arg Gln Gly Gln Val Arg Ile Ile Ser Asn Asp Val
  325                               330                               335

Cys Asn Ala Pro His Ser Tyr Asn Gly Ala Ile Leu Ser Gly Met Leu
  340                               345                               350

Cys Ala Gly Val Pro Gln Gly Gly Val Asp Ala Cys Gln Gly Asp Ser
  355                               360                               365

Gly Gly Pro Leu Val Gln Glu Asp Ser Arg Arg Leu Trp Phe Ile Val
  370                               375                               380

Gly Ile Val Ser Trp Gly Asp Gln Cys Gly Leu Pro Asp Lys Pro Gly
385                               390                               395                               400

Val Tyr Thr Arg Val Thr Ala Tyr Leu Asp Trp Ile Arg Gln Gln Thr
  405                               410                               415
    
```

Gly Ile

```

<210> SEQ ID NO 67
<211> LENGTH: 1783
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (246)...(1499)
<223> OTHER INFORMATION: Nucleotide sequence encoding human hepsin
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank M18930
<309> DATABASE ENTRY DATE: 1993-06-11
    
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<400> SEQUENCE: 67

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tcgagcccg tttccaggga ccctacctga gggcccacag gtgaggcagc ctggcctagc      60
aggccccacg ccaccgcctc tgctccagg cgcgccgctg ctgcggggac accatgctcc      120
tgcccaggcc tggagactga cccgaccccg gcactacctc gaggtccgc ccccacctgc      180
    
```

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tggaccccag ggtccccacc tggcccagga ggtcagccag ggaatcatta acaagaggca	240
gtgac atg gcg cag aag gag ggt ggc cgg act gtg cca tgc tgc tcc aga	290
Met Ala Gln Lys Glu Gly Gly Arg Thr Val Pro Cys Cys Ser Arg	
1 5 10 15	
ccc aag gtg gca gct ctc act gcg ggg acc ctg cta ctt ctg aca gcc	338
Pro Lys Val Ala Ala Leu Thr Ala Gly Thr Leu Leu Leu Leu Thr Ala	
20 25 30	
atc ggg gcg gca tcc tgg gcc att gtg gct gtt ctc ctc agg agt gac	386
Ile Gly Ala Ala Ser Trp Ala Ile Val Ala Val Leu Leu Arg Ser Asp	
35 40 45	
cag gag ccg ctg tac cca gtg cag gtc agc tct gcg gac gct cgg ctc	434
Gln Glu Pro Leu Tyr Pro Val Gln Val Ser Ser Ala Asp Ala Arg Leu	
50 55 60	
atg gtc ttt gac aag acg gaa ggg acg tgg cgg ctg ctg tgc tcc tcg	482
Met Val Phe Asp Lys Thr Glu Gly Thr Trp Arg Leu Leu Cys Ser Ser	
65 70 75	
cgc tcc aac gcc agg gta gcc gga ctc agc tgc gag gag atg gcc ttc	530
Arg Ser Asn Ala Arg Val Ala Gly Leu Ser Cys Glu Glu Met Gly Phe	
80 85 90 95	
ctc agg gca ctg acc cac tcc gag ctg gac gtg cga acg gcg gcc gcc	578
Leu Arg Ala Leu Thr His Ser Glu Leu Asp Val Arg Thr Ala Gly Ala	
100 105 110	
aat ggc acg tcg ggc ttc ttc tgt gtg gac gag ggg agg ctg ccc cac	626
Asn Gly Thr Ser Gly Phe Phe Cys Val Asp Glu Gly Arg Leu Pro His	
115 120 125	
acc cag agg ctg ctg gag gtc atc tcc gtg tgt gat tgc ccc aga gcc	674
Thr Gln Arg Leu Leu Glu Val Ile Ser Val Cys Asp Cys Pro Arg Gly	
130 135 140	
cgt ttc ttg gcc gcc atc tgc caa gac tgt ggc cgc agg aag ctg ccc	722
Arg Phe Leu Ala Ala Ile Cys Gln Asp Cys Gly Arg Arg Lys Leu Pro	
145 150 155	
gtg gac gcg atc gtg gga gcc cgg gac acc agc ttg gcc cgg tgg ccg	770
Val Asp Arg Ile Val Gly Gly Arg Asp Thr Ser Leu Gly Arg Trp Pro	
160 165 170 175	
tgg caa gtc agc ctt cgc tat gat gga gca cac ctc tgt ggg gga tcc	818
Trp Gln Val Ser Leu Arg Tyr Asp Gly Ala His Leu Cys Gly Gly Ser	
180 185 190	
ctg ctc tcc ggg gac tgg gtg ctg aca gcc gcc cac tgc ttc ccg gag	866
Leu Leu Ser Gly Asp Trp Val Leu Thr Ala Ala His Cys Phe Pro Glu	
195 200 205	
cgg aac ccg gtc ctg tcc cga tgg cga gtg ttt gcc ggt gcc gtg gcc	914
Arg Asn Arg Val Leu Ser Arg Trp Arg Val Phe Ala Gly Ala Val Ala	
210 215 220	
cag gcc tct ccc cac ggt ctg cag ctg ggg gtg cag gct gtg gtc tac	962
Gln Ala Ser Pro His Gly Leu Gln Leu Gly Val Gln Ala Val Val Tyr	
225 230 235	
cac ggg gcc tat ctt ccc ttt cgg gac ccc aac agc gag gag aac agc	1010
His Gly Gly Tyr Leu Pro Phe Arg Asp Pro Asn Ser Glu Glu Asn Ser	
240 245 250 255	
aac gat att gcc ctg gtc cac ctc tcc agt ccc ctg ccc ctc aca gaa	1058
Asn Asp Ile Ala Leu Val His Leu Ser Ser Pro Leu Pro Leu Thr Glu	
260 265 270	
tac atc cag cct gtg tgc ctc cca gct gcc ggc cag gcc ctg gtg gat	1106
Tyr Ile Gln Pro Val Cys Leu Pro Ala Ala Gly Gln Ala Leu Val Asp	
275 280 285	
ggc aag atc tgt acc gtg acg ggc tgg ggc aac acg cag tac tat ggc	1154

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Gly	Lys	Ile	Cys	Thr	Val	Thr	Gly	Trp	Gly	Asn	Thr	Gln	Tyr	Tyr	Gly	
	290						295					300				
caa	cag	gcc	ggg	gta	ctc	cag	gag	gct	cga	gtc	ccc	ata	atc	agc	aat	1202
Gln	Gln	Ala	Gly	Val	Leu	Gln	Glu	Ala	Arg	Val	Pro	Ile	Ile	Ser	Asn	
	305					310					315					
gat	gtc	tgc	aat	ggc	gct	gac	ttc	tat	gga	aac	cag	atc	aag	ccc	aag	1250
Asp	Val	Cys	Asn	Gly	Ala	Asp	Phe	Tyr	Gly	Asn	Gln	Ile	Lys	Pro	Lys	
320				325					330					335		
atg	ttc	tgt	gct	ggc	tac	ccc	gag	ggt	ggc	att	gat	gcc	tgc	cag	ggc	1298
Met	Phe	Cys	Ala	Gly	Tyr	Pro	Glu	Gly	Gly	Ile	Asp	Ala	Cys	Gln	Gly	
			340					345					350			
gac	agc	ggt	ggt	ccc	ttt	gtg	tgt	gag	gac	agc	atc	tct	cgg	acg	cca	1346
Asp	Ser	Gly	Gly	Pro	Phe	Val	Cys	Glu	Asp	Ser	Ile	Ser	Arg	Thr	Pro	
		355						360					365			
cgt	tgg	cgg	ctg	tgt	ggc	att	gtg	agt	tgg	ggc	act	ggc	tgt	gcc	ctg	1394
Arg	Trp	Arg	Leu	Cys	Gly	Ile	Val	Ser	Trp	Gly	Thr	Gly	Cys	Ala	Leu	
	370					375						380				
gcc	cag	aag	cca	ggc	gtc	tac	acc	aaa	gtc	agt	gac	ttc	cgg	gag	tgg	1442
Ala	Gln	Lys	Pro	Gly	Val	Tyr	Thr	Lys	Val	Ser	Asp	Phe	Arg	Glu	Trp	
	385					390						395				
atc	ttc	cag	gcc	ata	aag	act	cac	tcc	gaa	gcc	agc	ggc	atg	gtg	acc	1490
Ile	Phe	Gln	Ala	Ile	Lys	Thr	His	Ser	Glu	Ala	Ser	Gly	Met	Val	Thr	
400				405					410					415		
cag	ctc	tga	ccggtggcct	ctcgtctg	gcgc	agcctccagg	gcccagggtg									1539
Gln	Leu	*														
atccccggtgg	tgggatccac	gctgggccga	ggatgggacg	ttttcttct	tgggcccgtt											1599
ccacaggtcc	aaggacacc	tcctccagg	gtcctctctt	ccacagtggc	gggcccactc											1659
agccccgaga	ccaccaacc	tcacctctct	gacccccatg	taaataattgt	tctgctgtct											1719
gggactcctg	tctaggtgcc	cctgatgatg	ggatgctctt	taaataataa	agatggtttt											1779
gatt																1783

<210> SEQ ID NO 68  
 <211> LENGTH: 417  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo Sapien

<400> SEQUENCE: 68

Met	Ala	Gln	Lys	Glu	Gly	Gly	Arg	Thr	Val	Pro	Cys	Cys	Ser	Arg	Pro
1			5						10					15	
Lys	Val	Ala	Ala	Leu	Thr	Ala	Gly	Thr	Leu	Leu	Leu	Leu	Thr	Ala	Ile
		20					25						30		
Gly	Ala	Ala	Ser	Trp	Ala	Ile	Val	Ala	Val	Leu	Leu	Arg	Ser	Asp	Gln
	35					40						45			
Glu	Pro	Leu	Tyr	Pro	Val	Gln	Val	Ser	Ser	Ala	Asp	Ala	Arg	Leu	Met
	50					55				60					
Val	Phe	Asp	Lys	Thr	Glu	Gly	Thr	Trp	Arg	Leu	Leu	Cys	Ser	Ser	Arg
65				70					75					80	
Ser	Asn	Ala	Arg	Val	Ala	Gly	Leu	Ser	Cys	Glu	Glu	Met	Gly	Phe	Leu
			85					90						95	
Arg	Ala	Leu	Thr	His	Ser	Glu	Leu	Asp	Val	Arg	Thr	Ala	Gly	Ala	Asn
			100					105					110		
Gly	Thr	Ser	Gly	Phe	Phe	Cys	Val	Asp	Glu	Gly	Arg	Leu	Pro	His	Thr
	115						120						125		

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Gln Arg Leu Leu Glu Val Ile Ser Val Cys Asp Cys Pro Arg Gly Arg
 130                               135                               140

Phe Leu Ala Ala Ile Cys Gln Asp Cys Gly Arg Arg Lys Leu Pro Val
145                               150                               155                               160

Asp Arg Ile Val Gly Gly Arg Asp Thr Ser Leu Gly Arg Trp Pro Trp
                               165                               170                               175

Gln Val Ser Leu Arg Tyr Asp Gly Ala His Leu Cys Gly Gly Ser Leu
                               180                               185                               190

Leu Ser Gly Asp Trp Val Leu Thr Ala Ala His Cys Phe Pro Glu Arg
 195                               200                               205

Asn Arg Val Leu Ser Arg Trp Arg Val Phe Ala Gly Ala Val Ala Gln
 210                               215                               220

Ala Ser Pro His Gly Leu Gln Leu Gly Val Gln Ala Val Val Tyr His
225                               230                               235                               240

Gly Gly Tyr Leu Pro Phe Arg Asp Pro Asn Ser Glu Glu Asn Ser Asn
 245                               250                               255

Asp Ile Ala Leu Val His Leu Ser Ser Pro Leu Pro Leu Thr Glu Tyr
 260                               265                               270

Ile Gln Pro Val Cys Leu Pro Ala Ala Gly Gln Ala Leu Val Asp Gly
 275                               280                               285

Lys Ile Cys Thr Val Thr Gly Trp Gly Asn Thr Gln Tyr Tyr Gly Gln
 290                               295                               300

Gln Ala Gly Val Leu Gln Glu Ala Arg Val Pro Ile Ile Ser Asn Asp
305                               310                               315                               320

Val Cys Asn Gly Ala Asp Phe Tyr Gly Asn Gln Ile Lys Pro Lys Met
 325                               330                               335

Phe Cys Ala Gly Tyr Pro Glu Gly Gly Ile Asp Ala Cys Gln Gly Asp
 340                               345                               350

Ser Gly Gly Pro Phe Val Cys Glu Asp Ser Ile Ser Arg Thr Pro Arg
 355                               360                               365

Trp Arg Leu Cys Gly Ile Val Ser Trp Gly Thr Gly Cys Ala Leu Ala
 370                               375                               380

Gln Lys Pro Gly Val Tyr Thr Lys Val Ser Asp Phe Arg Glu Trp Ile
385                               390                               395                               400

Phe Gln Ala Ile Lys Thr His Ser Glu Ala Ser Gly Met Val Thr Gln
 405                               410                               415
    
```

Leu

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<210> SEQ ID NO 69
<211> LENGTH: 2479
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (57)...(1535)
<223> OTHER INFORMATION: Nucleotide sequence encoding human serine
    protease
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank U75329
<309> DATABASE ENTRY DATE: 1997-10-10

<400> SEQUENCE: 69
    
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gtcatattga acattccaga tacctatcat tactcgatgc tgttgataac agcaag atg      59
Met
1
    
```

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gct ttg aac tca ggg tca cca cca gct att gga cct tac tat gaa aac	107
Ala Leu Asn Ser Gly Ser Pro Pro Ala Ile Gly Pro Tyr Tyr Glu Asn	
5 10 15	
cat gga tac caa ccg gaa aac ccc tat ccc gca cag ccc act gtg gtc	155
His Gly Tyr Gln Pro Glu Asn Pro Tyr Pro Ala Gln Pro Thr Val Val	
20 25 30	
ccc act gtc tac gag gtg cat ccg gct cag tac tac ccg tcc ccc gtg	203
Pro Thr Val Tyr Glu Val His Pro Ala Gln Tyr Tyr Pro Ser Pro Val	
35 40 45	
ccc cag tac gcc ccg agg gtc ctg acg cag gct tcc aac ccc gtc gtc	251
Pro Gln Tyr Ala Pro Arg Val Leu Thr Gln Ala Ser Asn Pro Val Val	
50 55 60 65	
tgc acg cag ccc aaa tcc cca tcc ggg aca gtg tgc acc tca aag act	299
Cys Thr Gln Pro Lys Ser Pro Ser Gly Thr Val Cys Thr Ser Lys Thr	
70 75 80	
aag aaa gca ctg tgc atc acc ttg acc ctg ggg acc ttc ctc gtg gga	347
Lys Lys Ala Leu Cys Ile Thr Leu Thr Leu Gly Thr Phe Leu Val Gly	
85 90 95	
gct gcg ctg gcc gct ggc cta ctc tgg aag ttc atg ggc agc aag tgc	395
Ala Ala Leu Ala Ala Gly Leu Trp Lys Phe Met Gly Ser Lys Cys	
100 105 110	
tcc aac tct ggg ata gag tgc gac tcc tca ggt acc tgc atc aac ccc	443
Ser Asn Ser Gly Ile Glu Cys Asp Ser Ser Gly Thr Cys Ile Asn Pro	
115 120 125	
tct aac tgg tgt gat ggc gtg tca cac tgc ccc ggc ggg gag gac gag	491
Ser Asn Trp Cys Asp Gly Val Ser His Cys Pro Gly Gly Glu Asp Glu	
130 135 140 145	
aat cgg tgt gtt cgc ctc tac gga cca aac ttc atc ctt cag atg tac	539
Asn Arg Cys Val Arg Leu Tyr Gly Pro Asn Phe Ile Leu Gln Met Tyr	
150 155 160	
tca tct cag agg aag tcc tgg cac cct gtg tgc caa gac gac tgg aac	587
Ser Ser Gln Arg Lys Ser Trp His Pro Val Cys Gln Asp Asp Trp Asn	
165 170 175	
gag aac tac ggg ccg gcg gcc tgc agg gac atg ggc tat aag aat aat	635
Glu Asn Tyr Gly Arg Ala Ala Cys Arg Asp Met Gly Tyr Lys Asn Asn	
180 185 190	
ttt tac tct agc caa gga ata gtg gat gac agc gga tcc acc agc ttt	683
Phe Tyr Ser Ser Gln Gly Ile Val Asp Asp Ser Gly Ser Thr Ser Phe	
195 200 205	
atg aaa ctg aac aca agt gcc ggc aat gtc gat atc tat aaa aaa ctg	731
Met Lys Leu Asn Thr Ser Ala Gly Asn Val Asp Ile Tyr Lys Lys Leu	
210 215 220 225	
tac cac agt gat gcc tgt tct tca aaa gca gtg gtt tct tta cgc tgt	779
Tyr His Ser Asp Ala Cys Ser Ser Lys Ala Val Val Ser Leu Arg Cys	
230 235 240	
tta gcc tgc ggg gtc aac ttg aac tca agc cgc cag agc agg atc gtg	827
Leu Ala Cys Gly Val Asn Leu Asn Ser Ser Arg Gln Ser Arg Ile Val	
245 250 255	
ggc ggt gag agc gcg ctc ccg ggg gcc tgg ccc tgg cag gtc agc ctg	875
Gly Gly Glu Ser Ala Leu Pro Gly Ala Trp Pro Trp Gln Val Ser Leu	
260 265 270	
cac gtc cag aac gtc cac gtg tgc gga ggc tcc atc atc acc ccc gag	923
His Val Gln Asn Val His Val Cys Gly Gly Ser Ile Ile Thr Pro Glu	
275 280 285	
tgg atc gtg aca gcc gcc cac tgc gtg gaa aaa cct ctt aac aat cca	971
Trp Ile Val Thr Ala Ala His Cys Val Glu Lys Pro Leu Asn Asn Pro	
290 295 300 305	



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tgggggaaat caaggatgct cagttaagc tacactgttt ccatgttatg tttctacaca 2445

ttgatggtgg tgacctgag ttcaaagcca tctt 2479

<210> SEQ ID NO 70

<211> LENGTH: 492

<212> TYPE: PRT

<213> ORGANISM: Homo sapien

<400> SEQUENCE: 70

Met Ala Leu Asn Ser Gly Ser Pro Pro Ala Ile Gly Pro Tyr Tyr Glu  
1 5 10 15

Asn His Gly Tyr Gln Pro Glu Asn Pro Tyr Pro Ala Gln Pro Thr Val  
20 25 30

Val Pro Thr Val Tyr Glu Val His Pro Ala Gln Tyr Tyr Pro Ser Pro  
35 40 45

Val Pro Gln Tyr Ala Pro Arg Val Leu Thr Gln Ala Ser Asn Pro Val  
50 55 60

Val Cys Thr Gln Pro Lys Ser Pro Ser Gly Thr Val Cys Thr Ser Lys  
65 70 75 80

Thr Lys Lys Ala Leu Cys Ile Thr Leu Thr Leu Gly Thr Phe Leu Val  
85 90 95

Gly Ala Ala Leu Ala Ala Gly Leu Leu Trp Lys Phe Met Gly Ser Lys  
100 105 110

Cys Ser Asn Ser Gly Ile Glu Cys Asp Ser Ser Gly Thr Cys Ile Asn  
115 120 125

Pro Ser Asn Trp Cys Asp Gly Val Ser His Cys Pro Gly Gly Glu Asp  
130 135 140

Glu Asn Arg Cys Val Arg Leu Tyr Gly Pro Asn Phe Ile Leu Gln Met  
145 150 155 160

Tyr Ser Ser Gln Arg Lys Ser Trp His Pro Val Cys Gln Asp Asp Trp  
165 170 175

Asn Glu Asn Tyr Gly Arg Ala Ala Cys Arg Asp Met Gly Tyr Lys Asn  
180 185 190

Asn Phe Tyr Ser Ser Gln Gly Ile Val Asp Asp Ser Gly Ser Thr Ser  
195 200 205

Phe Met Lys Leu Asn Thr Ser Ala Gly Asn Val Asp Ile Tyr Lys Lys  
210 215 220

Leu Tyr His Ser Asp Ala Cys Ser Ser Lys Ala Val Val Ser Leu Arg  
225 230 235 240

Cys Leu Ala Cys Gly Val Asn Leu Asn Ser Ser Arg Gln Ser Arg Ile  
245 250 255

Val Gly Gly Glu Ser Ala Leu Pro Gly Ala Trp Pro Trp Gln Val Ser  
260 265 270

Leu His Val Gln Asn Val His Val Cys Gly Gly Ser Ile Ile Thr Pro  
275 280 285

Glu Trp Ile Val Thr Ala Ala His Cys Val Glu Lys Pro Leu Asn Asn  
290 295 300

Pro Trp His Trp Thr Ala Phe Ala Gly Ile Leu Arg Gln Ser Phe Met  
305 310 315 320

Phe Tyr Gly Ala Gly Tyr Gln Val Gln Lys Val Ile Ser His Pro Asn  
325 330 335

Tyr Asp Ser Lys Thr Lys Asn Asn Asp Ile Ala Leu Met Lys Leu Gln

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340										345					350					
Lys	Pro	Leu	Thr	Phe	Asn	Asp	Leu	Val	Lys	Pro	Val	Cys	Leu	Pro	Asn					
		355						360				365								
Pro	Gly	Met	Met	Leu	Gln	Pro	Glu	Gln	Leu	Cys	Trp	Ile	Ser	Gly	Trp					
		370						375				380								
Gly	Ala	Thr	Glu	Glu	Lys	Gly	Lys	Thr	Ser	Glu	Val	Leu	Asn	Ala	Ala					
	385				390							395			400					
Lys	Val	Leu	Leu	Ile	Glu	Thr	Gln	Arg	Cys	Asn	Ser	Arg	Tyr	Val	Tyr					
				405					410					415						
Asp	Asn	Leu	Ile	Thr	Pro	Ala	Met	Ile	Cys	Ala	Gly	Phe	Leu	Gln	Gly					
		420							425					430						
Asn	Val	Asp	Ser	Cys	Gln	Gly	Asp	Ser	Gly	Gly	Pro	Leu	Val	Thr	Ser					
		435						440				445								
Asn	Asn	Asn	Ile	Trp	Trp	Leu	Ile	Gly	Asp	Thr	Ser	Trp	Gly	Ser	Gly					
		450					455					460								
Cys	Ala	Lys	Ala	Tyr	Arg	Pro	Gly	Val	Tyr	Gly	Asn	Val	Met	Val	Phe					
	465				470							475			480					
Thr	Asp	Trp	Ile	Tyr	Arg	Gln	Met	Lys	Ala	Asn	Gly									
				485					490											
<210> SEQ ID NO 71																				
<211> LENGTH: 2079																				
<212> TYPE: DNA																				
<213> ORGANISM: Homo sapien																				
<220> FEATURE:																				
<221> NAME/KEY: CDS																				
<222> LOCATION: (251)...(1522)																				
<223> OTHER INFORMATION: Nucleotide sequence encoding transmembrane protease, serine 4 (TMPRSS4)																				
<300> PUBLICATION INFORMATION:																				
<308> DATABASE ACCESSION NUMBER: GenBank NM016425																				
<309> DATABASE ENTRY DATE: 2000-11-06																				
<400> SEQUENCE: 71																				
gagaggcagc agcttggtca gcggacaagg atgctgggcg tgagggacca aggcctgccc															60					
tgcactcggg cctcctccag ccagtgctga ccagggactt ctgacctgct ggccagccag															120					
gacctgtgtg gggaggccct cctgtgcct tggggtgaca atctcagctc caggctacag															180					
ggagaccggg aggatcacag agccagcatg gtacaggatc ctgacagtga tcaacctctg															240					
aacagcctcg atg tca aac ccc tgc gca aac ccc gta tcc cca tgg aga															289					
Met Ser Asn Pro Cys Ala Asn Pro Val Ser Pro Trp Arg																				
1 5 10																				
cct tca gaa agt gtg ggg atc ccc atc atc ata gca cta ctg agc ctg															337					
Pro Ser Glu Ser Val Gly Ile Pro Ile Ile Ile Ala Leu Leu Ser Leu																				
15 20 25																				
gcg agt atc atc att gtg gtt gtc ctc atc aag gtg att ctg gat aaa															385					
Ala Ser Ile Ile Ile Val Val Leu Ile Lys Val Ile Leu Asp Lys																				
30 35 40 45																				
tac tac ttc ctc tgc ggg cag cct ctc cac ttc atc ccg agg aag cag															433					
Tyr Tyr Phe Leu Cys Gly Gln Pro Leu His Phe Ile Pro Arg Lys Gln																				
50 55 60																				
ctg tgt gac gga gag ctg gac tgt ccc ttg ggg gag gac gag gag cac															481					
Leu Cys Asp Gly Glu Leu Asp Cys Pro Leu Gly Glu Asp Glu Glu His																				
65 70 75																				
tgt gtc aag agc ttc ccc gaa ggg cct gca gtg gca gtc cgc ctc tcc															529					
Cys Val Lys Ser Phe Pro Glu Gly Pro Ala Val Ala Val Arg Leu Ser																				
80 85 90																				

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aag gac cga tcc aca ctg cag gtg ctg gac tcg gcc aca ggg aac tgg	577
Lys Asp Arg Ser Thr Leu Gln Val Leu Asp Ser Ala Thr Gly Asn Trp	
95 100 105	
ttc tct gcc tgt ttc gac aac ttc aca gaa gct ctc gct gag aca gcc	625
Phe Ser Ala Cys Phe Asp Asn Phe Thr Glu Ala Leu Ala Glu Thr Ala	
110 115 120 125	
tgt agg cag atg ggc tac agc agc aaa ccc act ttc aga gct gtg gag	673
Cys Arg Gln Met Gly Tyr Ser Ser Lys Pro Thr Phe Arg Ala Val Glu	
130 135 140	
att ggc cca gac cag gat ctg gat gtt gtt gaa atc aca gaa aac agc	721
Ile Gly Pro Asp Gln Asp Leu Asp Val Val Glu Ile Thr Glu Asn Ser	
145 150 155	
cag gag ctt cgc atg cgg aac tca agt ggg ccc tgt ctc tca ggc tcc	769
Gln Glu Leu Arg Met Arg Asn Ser Ser Gly Pro Cys Leu Ser Gly Ser	
160 165 170	
ctg gtc tcc ctg cac tgt ctt gcc tgt ggg aag agc ctg aag acc ccc	817
Leu Val Ser Leu His Cys Leu Ala Cys Gly Lys Ser Leu Lys Thr Pro	
175 180 185	
cgt gtg gtg ggt ggg gag gag gcc tct gtg gat tct tgg cct tgg cag	865
Arg Val Val Gly Gly Glu Glu Ala Ser Val Asp Ser Trp Pro Trp Gln	
190 195 200 205	
gtc agc atc cag tac gac aaa cag cac gtc tgt gga ggg agc atc ctg	913
Val Ser Ile Gln Tyr Asp Lys Gln His Val Cys Gly Gly Ser Ile Leu	
210 215 220	
gac ccc cac tgg gtc ctc acg gca gcc cac tgc ttc agg aaa cat acc	961
Asp Pro His Trp Val Leu Thr Ala Ala His Cys Phe Arg Lys His Thr	
225 230 235	
gat gtg ttc aac tgg aag gtg cgg gca ggc tca gac aaa ctg ggc agc	1009
Asp Val Phe Asn Trp Lys Val Arg Ala Gly Ser Asp Lys Leu Gly Ser	
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ttc cca tcc ctg gct gtg gcc aag atc atc atc att gaa ttc aac ccc	1057
Phe Pro Ser Leu Ala Val Ala Lys Ile Ile Ile Ile Glu Phe Asn Pro	
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atg tac ccc aaa gac aat gac atc gcc ctc atg aag ctg cag ttc cca	1105
Met Tyr Pro Lys Asp Asn Asp Ile Ala Leu Met Lys Leu Gln Phe Pro	
270 275 280 285	
ctc act ttc tca ggc aca gtc agg ccc atc tgt ctg ccc ttc ttt gat	1153
Leu Thr Phe Ser Gly Thr Val Arg Pro Ile Cys Leu Pro Phe Phe Asp	
290 295 300	
gag gag ctc act cca gcc acc cca ctc tgg atc att gga tgg ggc ttt	1201
Glu Glu Leu Thr Pro Ala Thr Pro Leu Trp Ile Ile Gly Trp Gly Phe	
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acg aag cag aat gga ggg aag atg tct gac ata ctg ctg cag gcg tca	1249
Thr Lys Gln Asn Gly Gly Lys Met Ser Asp Ile Leu Leu Gln Ala Ser	
320 325 330	
gtc cag gtc att gac agc aca cgg tgc aat gca gac gat gcg tac cag	1297
Val Gln Val Ile Asp Ser Thr Arg Cys Asn Ala Asp Asp Ala Tyr Gln	
335 340 345	
ggg gaa gtc acc gag aag atg atg tgt gca ggc atc ccg gaa ggg ggt	1345
Gly Glu Val Thr Glu Lys Met Met Cys Ala Gly Ile Pro Glu Gly Gly	
350 355 360 365	
gtg gac acc tgc cag ggt gac agt ggt ggg ccc ctg atg tac caa tct	1393
Val Asp Thr Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Tyr Gln Ser	
370 375 380	
gac cag tgg cat gtg gtg ggc atc gtt agc tgg ggc tat ggc tgc ggg	1441
Asp Gln Trp His Val Val Gly Ile Val Ser Trp Gly Tyr Gly Cys Gly	
385 390 395	

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ggc ccg agc acc cca gga gta tac acc aag gtc tca gcc tat ctc aac      1489
Gly Pro Ser Thr Pro Gly Val Tyr Thr Lys Val Ser Ala Tyr Leu Asn
      400                      405                      410

tgg atc tac aat gtc tgg aag gct gag ctg taa tgctgctgcc cctttgcagt    1542
Trp Ile Tyr Asn Val Trp Lys Ala Glu Leu *
      415                      420

gctgggagcc gcttccttcc tgcctgccc acctggggat ccccaaaagt cagacacaga    1602

gcaagagtcc ccttgggtac acccctctgc ccacagcctc agcatttctt ggagcagcaa    1662

agggcctcaa ttctgtgaag agaccctcgc agcccagagg cgcccagagg aagtcagcag    1722

ccctagctcg gccacacttg gtgctcccag catcccaggg agagacacag cccactgaac    1782

aaggtctcag gggatttctg aagccaagaa ggaactttcc cacactactg aatggaagca    1842

ggctgtcttg taaaagccca gatcactgtg ggctggagag gagaaggaaa gggctgtcgc    1902

cagccctgtc cgtcttcacc catcccgaag cctactagag caagaaacca gttgtaatat    1962

aaaatgcact gccctactgt tggatgact accgttacct actggtgtca ttgttattac    2022

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<210> SEQ ID NO 72
<211> LENGTH: 423
<212> TYPE: PRT
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 72
    
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  1          5          10          15

Ser Val Gly Ile Pro Ile Ile Ile Ala Leu Leu Ser Leu Ala Ser Ile
      20          25          30

Ile Ile Val Val Val Leu Ile Lys Val Ile Leu Asp Lys Tyr Tyr Phe
      35          40          45

Leu Cys Gly Gln Pro Leu His Phe Ile Pro Arg Lys Gln Leu Cys Asp
      50          55          60

Gly Glu Leu Asp Cys Pro Leu Gly Glu Asp Glu Glu His Cys Val Lys
      65          70          75          80

Ser Phe Pro Glu Gly Pro Ala Val Ala Val Arg Leu Ser Lys Asp Arg
      85          90          95

Ser Thr Leu Gln Val Leu Asp Ser Ala Thr Gly Asn Trp Phe Ser Ala
      100          105          110

Cys Phe Asp Asn Phe Thr Glu Ala Leu Ala Glu Thr Ala Cys Arg Gln
      115          120          125

Met Gly Tyr Ser Ser Lys Pro Thr Phe Arg Ala Val Glu Ile Gly Pro
      130          135          140

Asp Gln Asp Leu Asp Val Val Glu Ile Thr Glu Asn Ser Gln Glu Leu
      145          150          155          160

Arg Met Arg Asn Ser Ser Gly Pro Cys Leu Ser Gly Ser Leu Val Ser
      165          170          175

Leu His Cys Leu Ala Cys Gly Lys Ser Leu Lys Thr Pro Arg Val Val
      180          185          190

Gly Gly Glu Glu Ala Ser Val Asp Ser Trp Pro Trp Gln Val Ser Ile
      195          200          205

Gln Tyr Asp Lys Gln His Val Cys Gly Gly Ser Ile Leu Asp Pro His
      210          215          220
    
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Trp Val Leu Thr Ala Ala His Cys Phe Arg Lys His Thr Asp Val Phe  
225 230 235 240

Asn Trp Lys Val Arg Ala Gly Ser Asp Lys Leu Gly Ser Phe Pro Ser  
245 250 255

Leu Ala Val Ala Lys Ile Ile Ile Ile Glu Phe Asn Pro Met Tyr Pro  
260 265 270

Lys Asp Asn Asp Ile Ala Leu Met Lys Leu Gln Phe Pro Leu Thr Phe  
275 280 285

Ser Gly Thr Val Arg Pro Ile Cys Leu Pro Phe Phe Asp Glu Glu Leu  
290 295 300

Thr Pro Ala Thr Pro Leu Trp Ile Ile Gly Trp Gly Phe Thr Lys Gln  
305 310 315 320

Asn Gly Gly Lys Met Ser Asp Ile Leu Leu Gln Ala Ser Val Gln Val  
325 330 335

Ile Asp Ser Thr Arg Cys Asn Ala Asp Asp Ala Tyr Gln Gly Glu Val  
340 345 350

Thr Glu Lys Met Met Cys Ala Gly Ile Pro Glu Gly Gly Val Asp Thr  
355 360 365

Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Tyr Gln Ser Asp Gln Trp  
370 375 380

His Val Val Gly Ile Val Ser Trp Gly Tyr Gly Cys Gly Gly Pro Ser  
385 390 395 400

Thr Pro Gly Val Tyr Thr Lys Val Ser Ala Tyr Leu Asn Trp Ile Tyr  
405 410 415

Asn Val Trp Lys Ala Glu Leu  
420

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What is claimed is:

1. A substantially purified single chain polypeptide, comprising the protease domain of a type-II membrane-type serine protease (MTSP) or a catalytically active portion thereof, wherein:

the MTSP portion of the protein consists essentially of the protease domain of the MTSP or a catalytically active portion thereof.

2. The substantially purified polypeptide of claim 1, wherein the MTSP is not expressed endothelial cells.

3. The substantially purified polypeptide of claim 1, wherein the MTSP is not expressed on normal endothelial cells in vivo.

4. The substantially purified polypeptide of claim 1, wherein the MTSP is a human protein.

5. The substantially purified polypeptide of claim 1 that consists essentially of the protease domain of an MTSP or a catalytically active portion of the protease domain.

6. The substantially purified polypeptide of claim 1, wherein the expression and/or activity of the MTSP in tumor cells differs from its level of expression and/or activity in non-tumor cells.

7. The substantially purified polypeptide of claim 1, wherein the MTSP is detectable in a body fluid at a level that differs from its level in body fluids in a subject not having a tumor.

8. The substantially purified polypeptide of claim 1, wherein:

the MTSP is present in a tumor; and

a substrate or cofactor for the MTSP is expressed at levels that differ from a non-tumor cell in the same tissue.

9. The substantially purified polypeptide of claim 1, wherein: the MTSP exhibits altered substrate specificity in the tumor compared to its specificity in a non-tumor cell in the same tissue.

10. The substantially purified polypeptide of claim 1, wherein the MTSP has an N-terminus that comprises IVNG, ILGG, VGLL or ILGG.

11. The substantially purified polypeptide of claim 1, wherein the MTSP is selected from among MTSP1, MTSP3, MTSP4 and MTSP6.

12. The substantially purified polypeptide of claim 1, wherein the protease domain comprises the sequence of amino acids set forth as amino acids 615-855 of SEQ ID No. 2, as amino acids 205-437 of SEQ ID NO. 4, as the amino acids in SEQ ID No. 6, or as amino acids 217-443 in SEQ ID No. 12.

13. The substantially purified polypeptide of claim 1 that has at least about 40%, 60%, 80%, 90% or 95% sequence identity with a protease domain that comprises the sequence of amino acids set forth as amino acids 615-855 of SEQ ID No. 2, as amino acids 205-437 of SEQ ID NO. 4, as the amino acids in SEQ ID No. 6, or as amino acids 217-443 in SEQ ID No. 12.

14. A polypeptide of claim 1, wherein the protease domain portion is encoded by a nucleic acid molecule that hybrid-

izes under conditions of high stringency along its full length to a nucleic acid molecule comprising a sequence of nucleotides set forth in SEQ ID No: 1, 3, 5, 7, 9 or 11 or to a molecule that encodes the protein set forth in SEQ ID No: 2, 4, 6, 8, 10 or 12 or at least one domain thereof.

**15.** A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of claim 1.

**16.** A mutein of the polypeptide of claim 1, wherein:

up to about 90% of the amino acids are replaced with another amino acid;

and the resulting polypeptide is a single chain and has catalytic activity at least 10% of the unmutated polypeptide.

**17.** The mutein of claim 16, wherein up to about 95% of the amino acids are replaced.

**18.** The mutein of claim 16, wherein the resulting polypeptide is a single chain and has catalytic activity at least 50% of the unmutated polypeptide.

**19.** A mutein of the polypeptide of claim 1, wherein a free Cys in the protease domain is replaced with another amino acid, whereby the resulting polypeptide exhibits proteolytic activity.

**20.** A mutein of the polypeptide of claim 1, wherein a free Cys in the protease domain is replaced with a serine.

**21.** A vector comprising the nucleic acid molecule of claim 15.

**22.** The vector of claim 21 that is an expression vector.

**23.** The vector of claim 21 that includes a sequence of nucleotides that directs secretion of any protein encoded by a sequence of nucleotides operatively linked thereto.

**24.** The vector of claim 21 that is a *Pichia* vector or an *E. coli* vector.

**25.** A cell, comprising the vector of claim 21.

**26.** The cell of claim 25 that is a prokaryotic cell.

**27.** The cells of claim 25 that is a eukaryotic cell.

**28.** The cell of claim 25 that is selected from among a bacterial cell, a yeast cell, a yeast cell, a plant cell, an insect cell and an animal cell.

**29.** The cell of claim 25 that is a mammalian cell.

**30.** A method for producing a polypeptide that contains a protease domain of an MTSP, comprising:

culturing the cell of claim 25 under conditions whereby the encoded protein is expressed by the cell; and

recovering the expressed protein.

**31.** The method of claim 30, wherein the cell is a *pichia* cell and the protein is secreted into the culture medium.

**32.** An antisense nucleic acid molecule that comprises at least 14 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the protease domain of an MTSP of claim 1; or

comprises at least 16 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the protease domain of an MTSP of claim 1.

**33.** An antibody that specifically binds to the single chain form of a protease domain of the polypeptide of claim 1, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

**34.** The polypeptide of claim 1, wherein the MTSP is selected from among corin, MTSP1, enterpeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4.

**35.** A conjugate, comprising:

a) a protein of claim 1, and

b) a targeting agent linked to the protein directly or via a linker.

**36.** The conjugate of claim 35, wherein the targeting agent permits

i) affinity isolation or purification of the conjugate;

ii) attachment of the conjugate to a surface;

iii) detection of the conjugate; or

iv) targeted delivery to a selected tissue or cell.

**36.** A combination, comprising:

a) an inhibitor of the catalytic activity of the polypeptide of claim 1; and

b) another treatment or agent selected from anti-tumor and anti-angiogenic treatments or agents.

**37.** The combination of claim 36, wherein the inhibitor and the anti-tumor and/or anti-angiogenic agent are formulated in a single pharmaceutical composition or each is formulated in separate pharmaceutical compositions.

**38.** The combination of claim 36, wherein the inhibitor is selected from antibodies and antisense oligonucleotides.

**39.** A solid support comprising two or more polypeptides of claim 1 linked thereto either directly or via a linker.

**40.** The support of claim 39, wherein the polypeptides comprise an array.

**41.** The support of claim 39, wherein the polypeptides comprise a plurality of different MTSP protease domains.

**42.** A method for identifying compounds that modulate the protease activity of an MTSP, comprising:

contacting a polypeptide of claim 1 with a substrate proteolytically cleaved by the MTSP, and, either simultaneously, before or after, adding a test compound or plurality thereof;

measuring the amount of substrate cleaved in the presence of the test compound; and

selecting compounds that change the amount cleaved compared to a control, whereby compounds that modulate the activity of the MTSP are identified.

**43.** The method of claim 42, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

**44.** The method of claim 42, wherein a plurality of the test substances are screened simultaneously.

**45.** The method of claim 42, wherein the change in the amount cleaved is assessed by comparing the amount cleaved in the presence of the test compound with the amount in the absence of the test compound.

**46.** The method of claim 42, wherein a plurality of the test substances are screened for simultaneously.

**47.** The method of claim 42, wherein a plurality of the polypeptides are linked to a solid support, either directly or via a linker.

**48.** The method of claim 42, wherein the polypeptides comprise an array.

49. The method of claim 42, wherein the polypeptides comprise a plurality of different MTSP proteases.

50. A method of identifying a compound that specifically binds to a single chain protease domain of an MTSP, comprising:

contacting a polypeptide of claim 1 with a test compound or

plurality thereof under conditions conducive to binding thereof; and

identifying compounds that specifically bind to the MTSP single chain protease domain or compounds that inhibit binding of a compound known to bind to the MTSP single chain protease domain, wherein the known compound is contacted with the polypeptide before, simultaneously with or after the test compound.

51. The method of claim 50, wherein the polypeptide is linked either directly or indirectly via a linker to a solid support.

52. The method of claim 50, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

53. The method of claim 50, wherein a plurality of the test substances are screened for simultaneously.

54. The method of claim 51, wherein a plurality of the polypeptides are linked to a solid support.

55. A substantially purified membrane-type serine protease 3 (MTSP3).

56. The MTSP3 of claim 43 that is selected from the group consisting of:

a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 3;

a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 3;

a polypeptide that comprises the sequence of amino acids set forth as amino acids 205-437 of SEQ ID No. 4;

a polypeptide that comprises a sequence of amino acids having at least about 90% sequence identity with the sequence of amino acids set forth in SEQ ID No. 4; and

a polypeptide encoded by a splice variant of the sequence of nucleotides set forth in SEQ ID No. 3.

57. A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of claim 56.

58. A vector comprising the nucleic acid molecule of claim 57.

59. A cell, comprising the vector of claim 58.

60. The cell of claim 59 that expresses the MTSP3 on its surface.

61. The cell of claim 59 that is a prokaryotic cell.

62. The cells of claim 59 that is a eukaryotic cell.

63. The cell of claim 59 that is selected from among a bacterial cell, a yeast cell, a yeast cell, a plant cell, an insect cell and an animal cell.

64. The cell of claim 59 that is a mammalian cell.

65. The cell of claim 59, wherein the MTSP3 comprises the sequence of amino acids set forth in SEQ ID No. 4; or a sequence of amino acids encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 3; or a protein having at least about 90% sequence identity

with the sequence of amino acids set forth in SEQ ID No. 4 and retaining protease activity.

66. A method for producing an MTSP3, comprising:

culturing the cell of claim 59 under conditions whereby the encoded protein is expressed by the cell; and

recovering the expressed protein.

67. An antisense nucleic acid molecule that comprises at least 14 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the nucleic acid molecule of claim 57; or

comprises at least 16 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the nucleic acid molecule of claim 57.

68. An antibody that specifically binds to the MTSP of claim 57, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

69. A method for treating or preventing a neoplastic disease, in a mammal, comprising administering to a mammal an effective amount of an inhibitor of an MTSP3 of claim 55.

70. The method of claim 69, wherein the inhibitor is an antibody that specifically binds to the MTSP3, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

71. A substantially purified membrane-type serine protease 4 (MTSP4).

72. The substantially purified MTSP4 that is an MTSP4-L or an MTSP4-S.

73. The MTSP4 of claim 71 that is selected from the group consisting of:

a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 7 or 9;

a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 7 or 9;

a polypeptide that comprises the sequence of amino acids set forth in SEQ ID No. 6, 8 or 10; and

a polypeptide encoded by a splice variant of the sequence of nucleotides set forth in SEQ ID No. 7 or 9.

74. The MTSP4 of claim 73 that is an MTSP4-L or an MTSP4-S.

75. A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of claim 73.

76. A vector comprising the nucleic acid molecule of claim 74.

77. A cell, comprising the vector of claim 76.

78. The cell of claim 77 that expresses the MTSP4 on its surface.

79. The cell of claim 77 that is a prokaryotic cell.

80. The cells of claim 77 that is a eukaryotic cell.

81. The cell of claim 77 that is selected from among a bacterial cell, a yeast cell, a yeast cell, a plant cell, an insect cell and an animal cell.

82. The cell of claim 77 that is a mammalian cell.

83. The cell of claim 77, wherein the MTSP4 comprises the sequence of amino acids set forth in SEQ ID No. 6, 8 or 10; or a sequence of amino acids encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of amino acids set forth in SEQ ID No. 6, 8 or 10; or a protein having at least about 90% sequence identity

gency to the sequence of nucleotides set forth in SEQ ID No. 7 or 9; or a protein having at least about 90% sequence identity with the sequence of amino acids set forth in SEQ ID No. 8 or 10 and retaining protease activity.

**84.** A method for producing an MTSP4, comprising:

culturing the cell of claim 77 under conditions whereby the encoded protein is expressed by the cell; and

recovering the expressed protein.

**85.** An antisense nucleic acid molecule that comprises at least 14 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the nucleic acid molecule of claim 75 or

comprises at least 16 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the nucleic acid molecule of claim 75.

**86.** An antibody that specifically binds to the MTSP of claim 72, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

**87.** An antibody that specifically binds to the MTSP of claim 73, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

**88.** A method for treating or preventing a neoplastic disease, in a mammal, comprising administering to a mammal an effective amount of an inhibitor of an MTSP4 of claim 71.

**89.** The method of claim 88, wherein the inhibitor is an antibody that specifically binds to an MTSP4, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

**90.** A substantially purified membrane-type serine protease 6 (MTSP6) selected from the group consisting of:

a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 11;

a polypeptide encoded by a sequence of nucleotides that hybridizes along the full length thereof under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 11;

a polypeptide that comprises the sequence of amino acids set forth as amino acids 217-443 of SEQ ID No. 12;

a polypeptide encoded by a splice variant of the sequence of nucleotides set forth in SEQ ID No. 11.

**91.** A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of claim 90.

**92.** A vector comprising the nucleic acid molecule of claim 91.

**93.** A cell, comprising the vector of claim 92.

**94.** The cell of claim 93 that expresses the MTSP6 on its surface.

**95.** The cell of claim 93 that is a prokaryotic cell.

**96.** The cells of claim 93 that is a eukaryotic cell.

**97.** The cell of claim 93 that is selected from among a bacterial cell, a yeast cell, a yeast cell, a plant cell, an insect cell and an animal cell.

**98.** The cell of claim 93 that is a mammalian cell.

**99.** The cell of claim 93, wherein the MTSP6 comprises the sequence of amino acids set forth in SEQ ID No. 12; or a sequence of amino acids encoded by a sequence of

nucleotides that hybridizes along the full length thereof under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 12; or a protein having at least about 95% sequence identity with the sequence of amino acids set forth in SEQ ID No. 4 and retaining protease activity.

**100.** A method for producing an MTSP6, comprising:

culturing the cell of claim 93 under conditions whereby the encoded protein is expressed by the cell; and

recovering the expressed protein.

**101.** An antisense nucleic acid molecule that comprises at least 14 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the nucleic acid molecule of claim 91; or

comprises at least 16 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the in the nucleic acid molecule of claim 91.

**102.** An antibody that specifically binds to the MTSP of claim 90, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

**103.** A method for treating or preventing a neoplastic disease, in a mammal, comprising administering to a mammal an effective amount of an inhibitor of an MTSP6 of claim 90.

**104.** The method of claim 103, wherein the inhibitor is an antibody that specifically binds to the MTSP6, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

**105.** A recombinant non-human animal, wherein an endogenous gene of an MTSP has been deleted or inactivated by homologous recombination or insertional mutagenesis of the animal or an ancestor thereof.

**106.** A recombinant non-human animal of claim 105, wherein the MTSP is an MTSP1, MTSP3, MTSP4 or MTSP6.

**107.** A conjugate, comprising:

- a) an MTSP3 or MTSP4 or an MTSP6 of claim 90; and
- b) a targeting agent linked to the protein directly or via a linker.

**108.** The conjugate of claim 106, wherein the targeting agent permits

- i) affinity isolation or purification of the conjugate;
- ii) attachment of the conjugate to a surface;
- iii) detection of the conjugate; or
- iv) targeted delivery to a selected tissue or cell.

**109.** A combination, comprising:

- a) an inhibitor of the catalytic activity of an MTSP3 or MTSP4 or MTSP6 of claim 90; and
- b) another treatment or agent selected from anti-tumor and anti-angiogenic treatments or agents.

**110.** The combination of claim 109, wherein the inhibitor and the anti-tumor and/or anti-angiogenic agent are formulated in a single pharmaceutical composition or each is formulated in separate pharmaceutical compositions.

**111.** The combination of claim 109, wherein the inhibitor is selected from antibodies and antisense oligonucleotides.

**112.** A solid support comprising two or more MTSP3 polypeptides and/or MTSP4 polypeptides and/or MTSP6 polypeptides of claim 90 linked thereto either directly or via a linker.

**113.** The support of claim 112, wherein the polypeptides comprise an array.

**114.** A method for identifying compounds that modulate the protease activity of an MTSP selected from MTSP3, MTSP4 or MTSP6 of claim 90, comprising:

contacting the MTSP with a substrate proteolytically cleaved by the MTSP, and, either simultaneously, before or after, adding a test compound or plurality thereof;

measuring the amount of substrate cleaved in the presence of the test compound; and

selecting compounds that change the amount cleaved compared to a control, whereby compounds that modulate the activity of the MTSP are identified.

**115.** The method of claim 114, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

**116.** The method of claim 114, wherein a plurality of the test substances are screened for simultaneously.

**117.** The method of claim 114, wherein the change in the amount cleaved is assessed by comparing the amount cleaved in the presence of the test compound with the amount in the absence of the test compound.

**118.** The method of claim 114, wherein a plurality of the test substances are screened for simultaneously.

**119.** The method of claim 118, wherein a plurality of the polypeptides are linked to a solid support.

**120.** A method of identifying a compound that specifically binds to a MTSP selected from MTSP3, MTSP4 and the MTSP6 of claim 90, comprising:

contacting the MTSP polypeptide with a test compound or plurality thereof under conditions conducive to binding thereof; and

identifying compounds that specifically binds to the MTSP.

**121.** A method of identifying a compound that specifically binds to a MTSP selected from MTSP3, MTSP4 and the MTSP6 of claim 90, comprising:

contacting the MTSP polypeptide with a test compound or plurality thereof under conditions conducive to binding thereof; and

identifying compounds that specifically binds to the MTSP.

**122.** The method of any claims 121, wherein the polypeptide is linked either directly or indirectly via a linker to a solid support.

**123.** The method of claim 121, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

**124.** The method of claim 121, wherein a plurality of the test substances are screened for simultaneously.

**125.** The method of claim 124, wherein a plurality of the polypeptides are linked to a solid support.

**126.** An MTSP6 polypeptide, comprising amino acids set forth as amino acids 46-55 in SEQ ID No. 12 and/or amino acids 368-394 of SEQ ID No. 12, and that is encoded by a sequence of nucleic acids that hybridizes under moderate stringency to nucleic acid encoding the polypeptide set forth in SEQ ID No. 12.

**127.** The polypeptide of claim 126 that comprises the amino acids set forth as amino acids 46-55 in SEQ ID No. 12 and/or amino acids 368-394 of SEQ ID No. 12, and that is encoded by a sequence of nucleic acids that hybridizes under high stringency along its full length or full length of the protease domain to nucleic acid encoding the polypeptide set forth in SEQ ID No. 12.

**128.** The polypeptide of claim 126, comprising the sequence of amino acids set forth in SEQ ID No. 12.

**129.** An isolated nucleic acid molecule, comprising a sequence of nucleic acids that encodes the polypeptide of claim 126.

**130.** A method for treating tumors, comprising administering a prodrug that is specifically cleaved by an MTSP, whereby, upon contact with a cell that expresses MTSP activity, the prodrug is converted into an active drug.

**131.** The method of claim 130, wherein the MTSP is selected from among an MTSP3, MTSP4 and an MTSP6 of claim 90.

**132.** The method of claim 130, wherein the MTSP is selected from among corin, enterpeptidase, human airway trypsin-like protease (HAT), MTSP1, TMRSS2, and TMRSS4.

**133.** A method of detecting neoplastic disease, comprising: detecting an MTSP3, MTSP4 or MTSP6 of claim 90 in a biological sample, wherein the amount detected differs from the amount in a subject who does not have neoplastic disease.

**134.** The method of claim 133, wherein the biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, cerebrospinal fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

**135.** The method of claim 133, wherein the extracellular domain of the MTSP3, MTSP4 or MTSP6 is in the sample.

\* \* \* \* \*

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摘要(译)

本文提供的是包含II型跨膜丝氨酸蛋白酶 ( MTSP ) 的蛋白酶结构域作为单链的多肽。提供了使用多肽鉴定调节MTSP的蛋白酶活性的化合物的方法。还提供了指定MTSP3和MTSP4的MTSP以及指定MTSP6的MTSP形式。

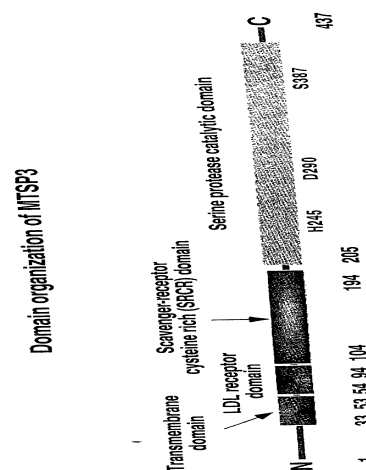


FIGURE 1