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O'Brien

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(54) **METHODS FOR THE EARLY DIAGNOSIS OF OVARIAN, BREAST AND LUNG CANCER**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

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(63) Continuation-in-part of application No. 09/039,211, filed on Mar. 14, 1998

(60) Provisional application No. 60/041,404, filed on Mar. 19, 1997, now abandoned.

(51) Int. Cl.⁷ **G01N 33/53**; C12Q 1/68; C07K 14/435

(52) U.S. Cl. **435/23**; 435/6; 435/7.1; 435/7.4; 435/7.7; 530/350; 536/23.5; 536/24.31

(58) Field of Search 435/6, 7.7, 7.4, 435/23, 7.1; 536/23.5, 24.31, 23.2; 530/350

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(57) **ABSTRACT**

The disclosed nucleic acid primer sets, used in combination with quantitative amplification (PCR) of tissue cDNA, can indicate the presence of specific proteases in a tissue sample. The detected proteases are themselves specifically overexpressed in certain cancers, and their presence may serve for early detection of associated ovarian and other malignancies, and for the design of interactive therapies for cancer treatment.

9 Claims, 19 Drawing Sheets

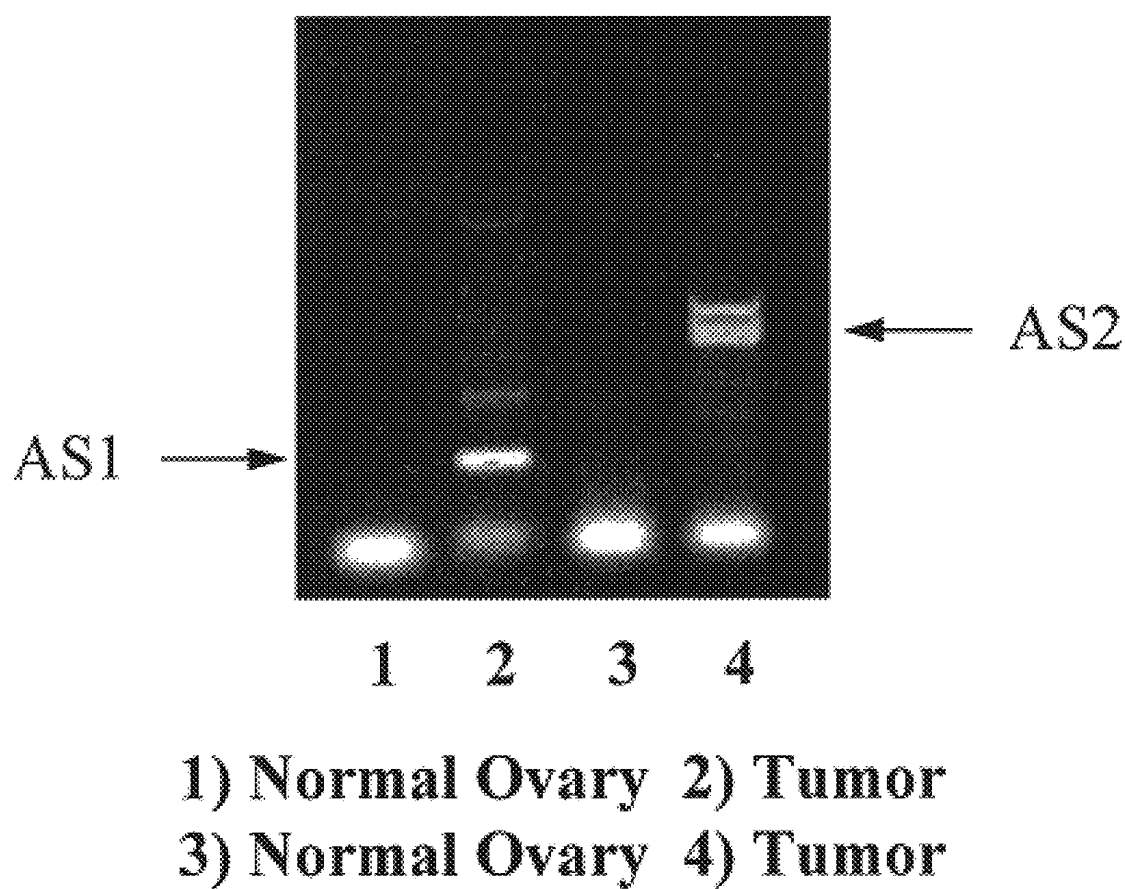


Fig. 1

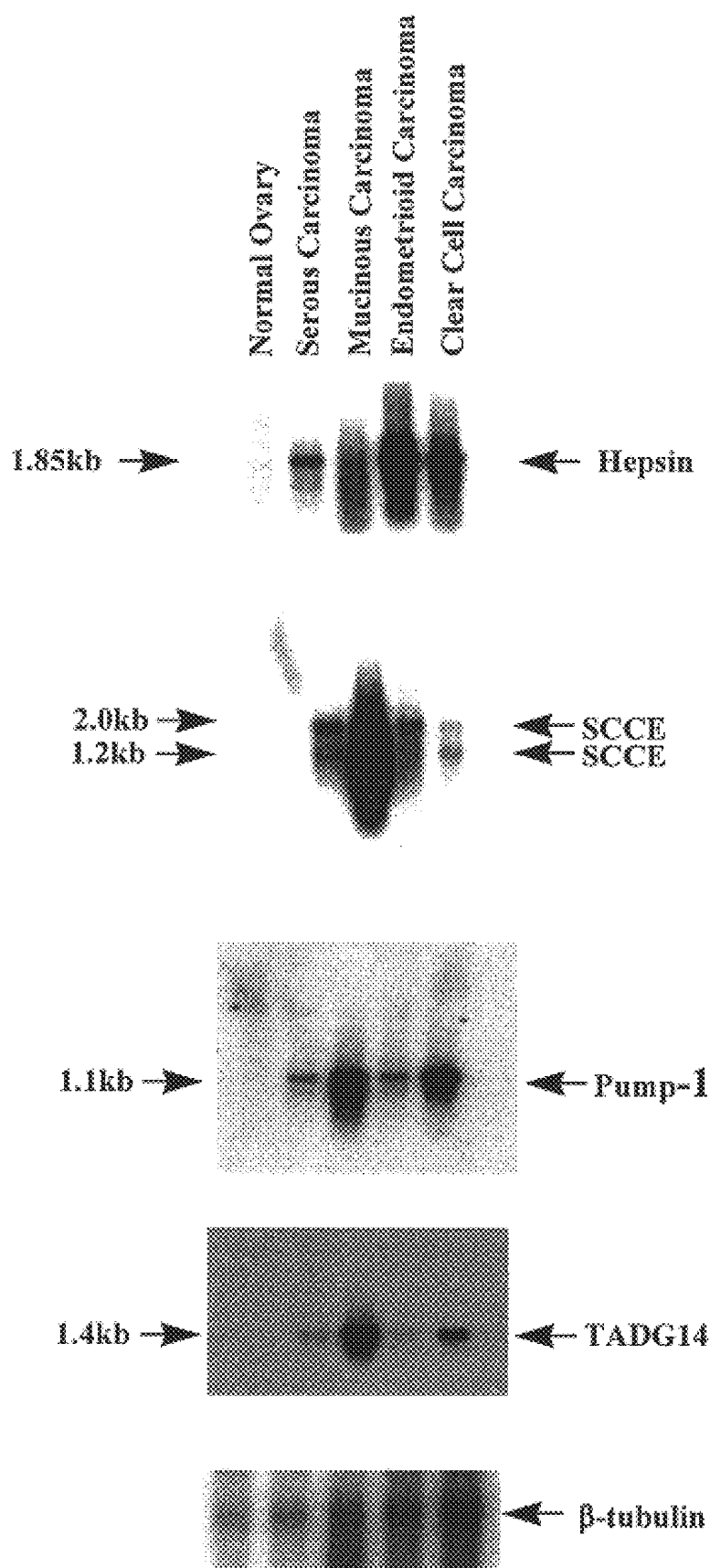
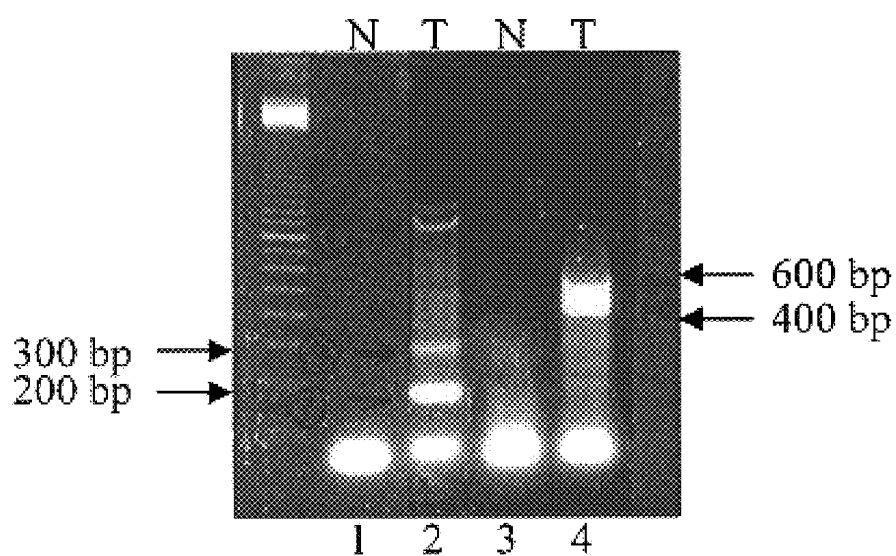
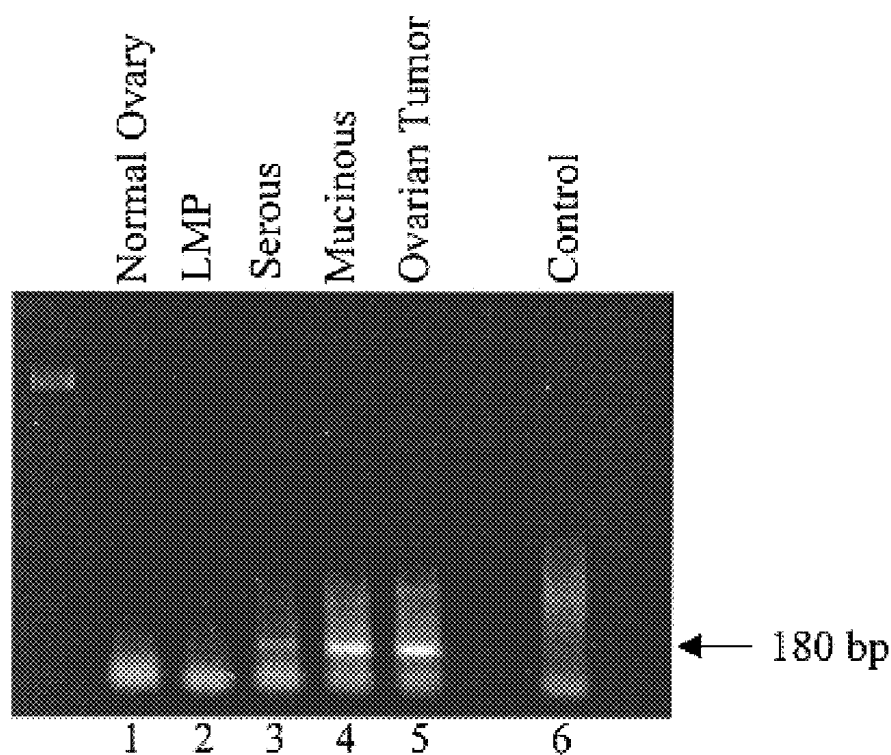


Fig. 2

**Fig. 3****Fig. 4**

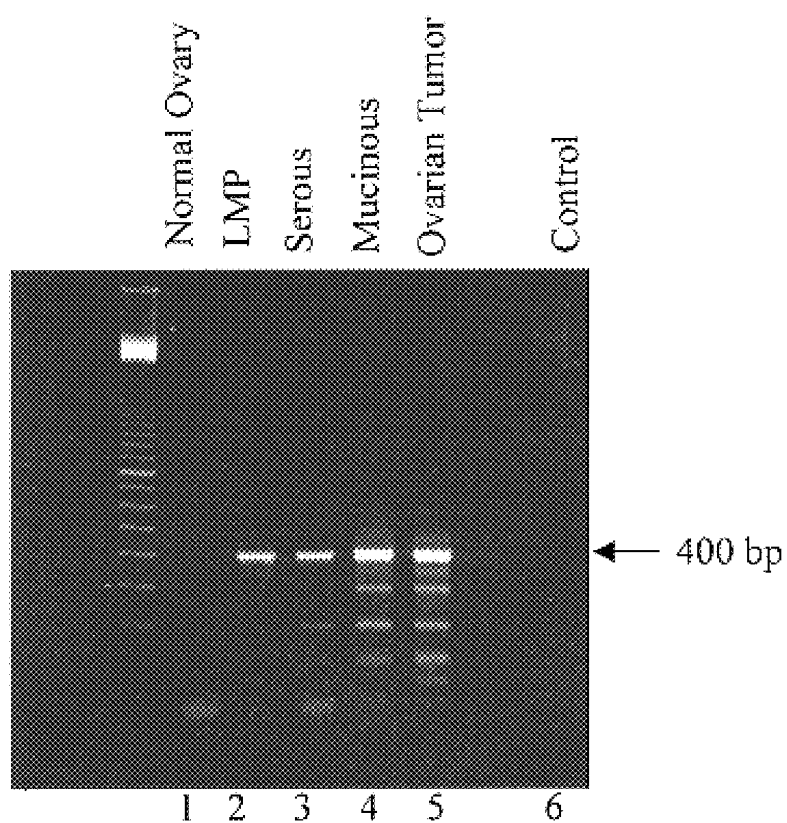


Fig. 5

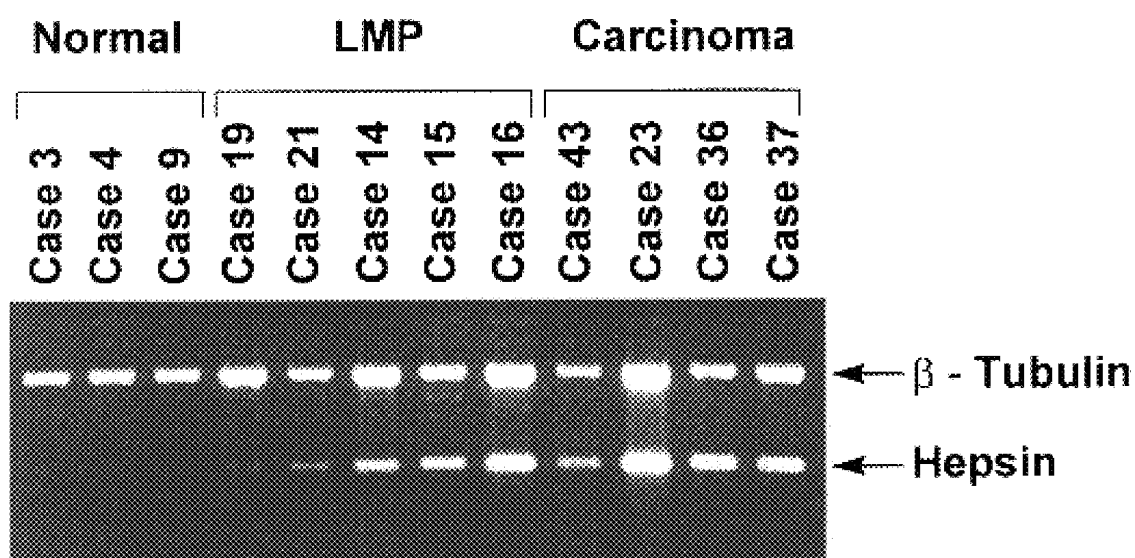


Fig. 6

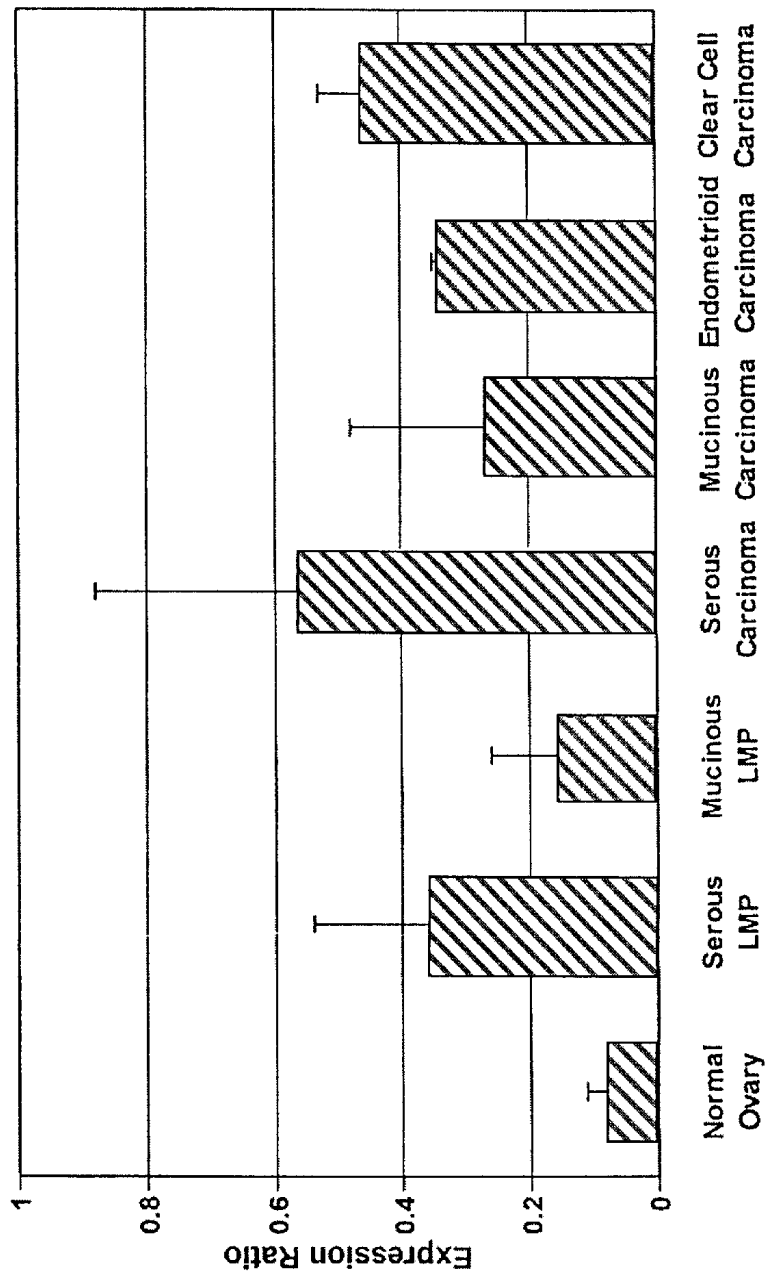


Fig. 7

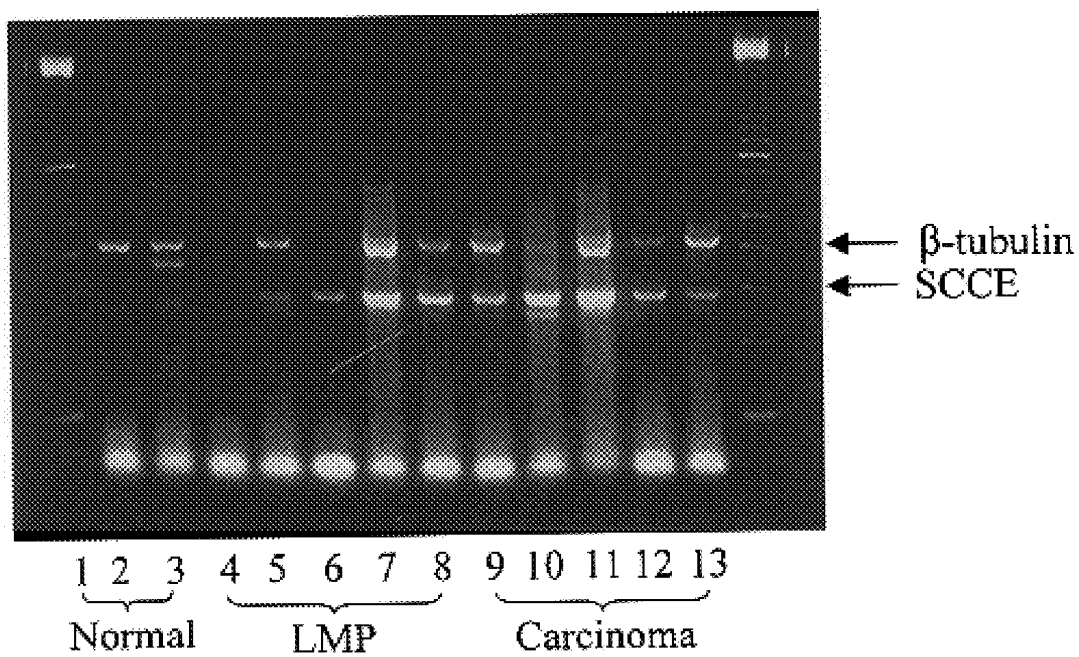


Fig. 8

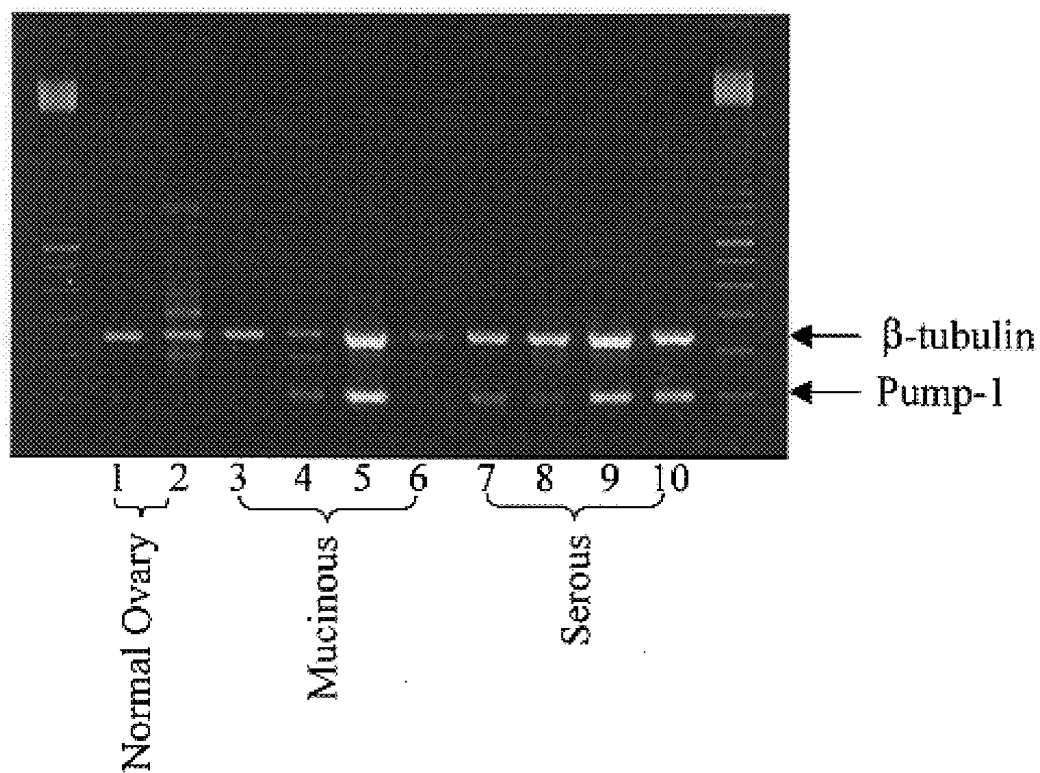


Fig. 9

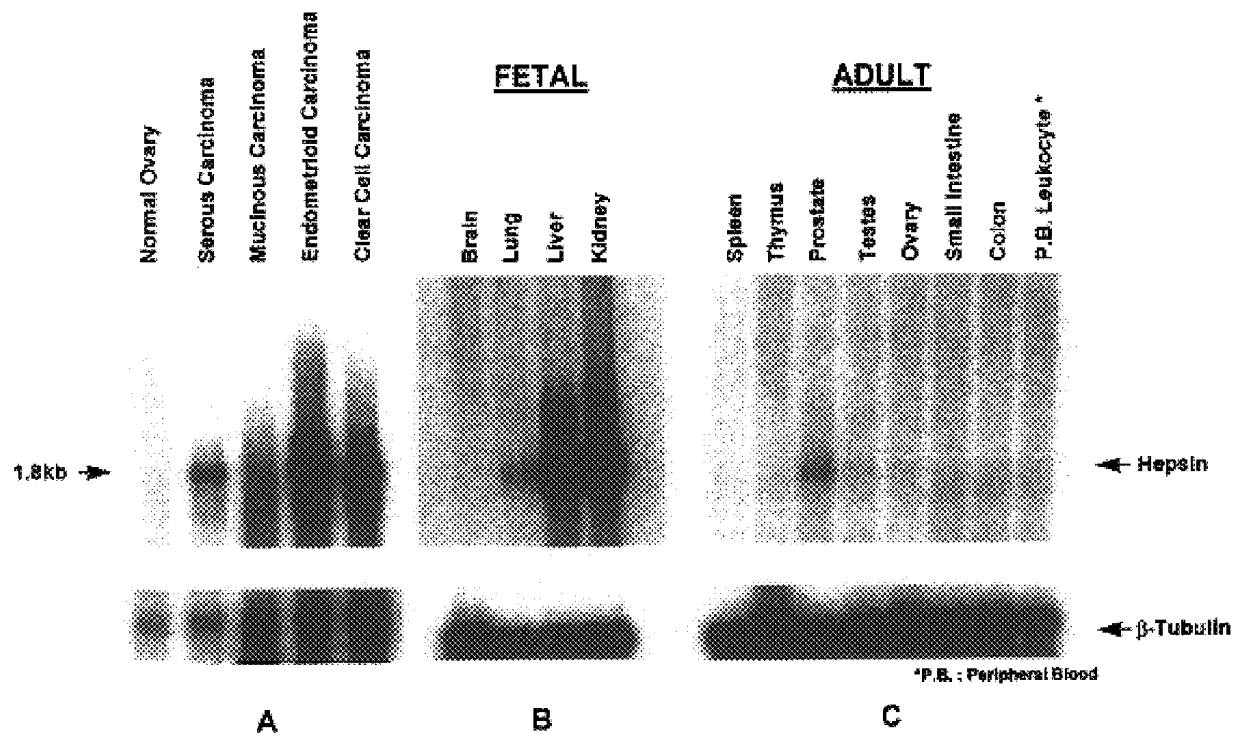


Fig. 10

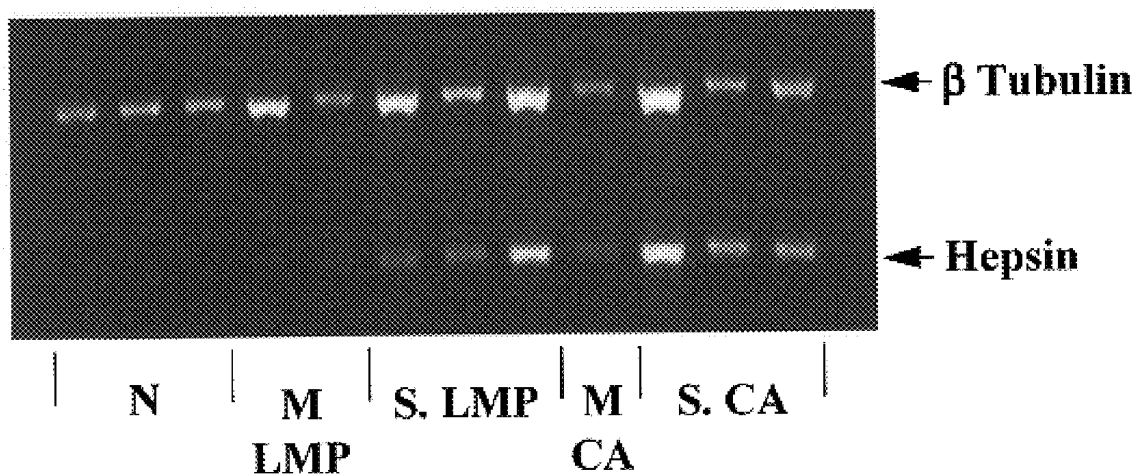


Fig. 11A

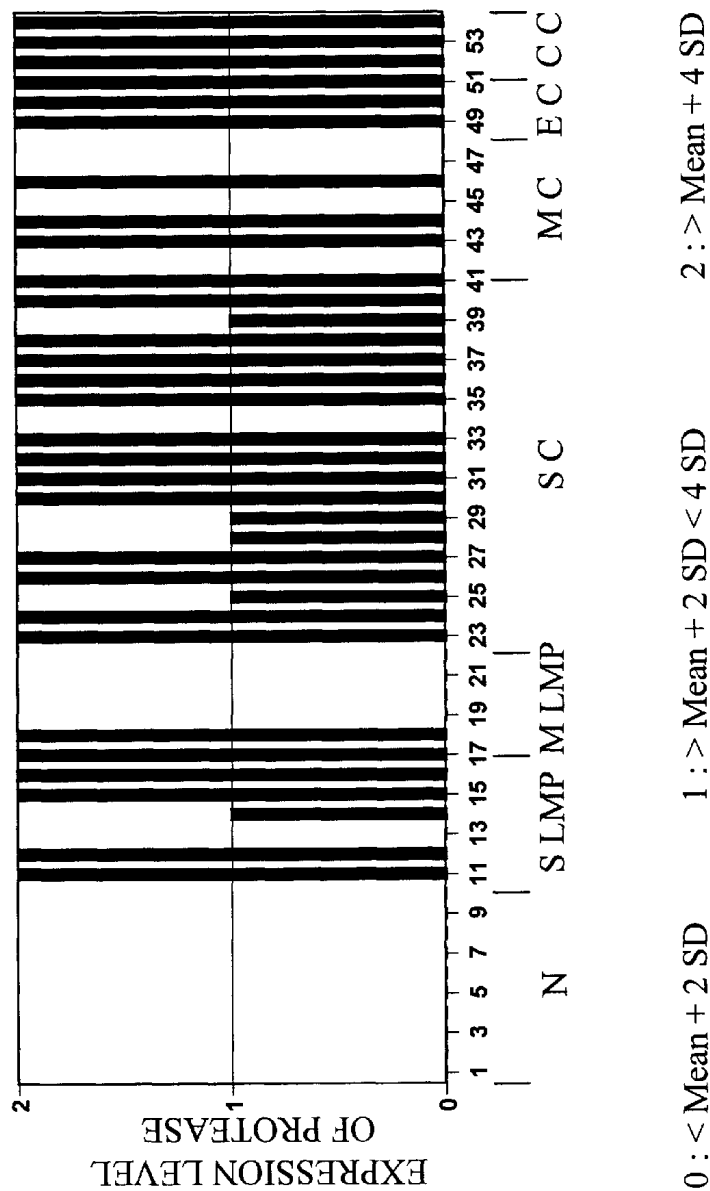


Fig. 11B

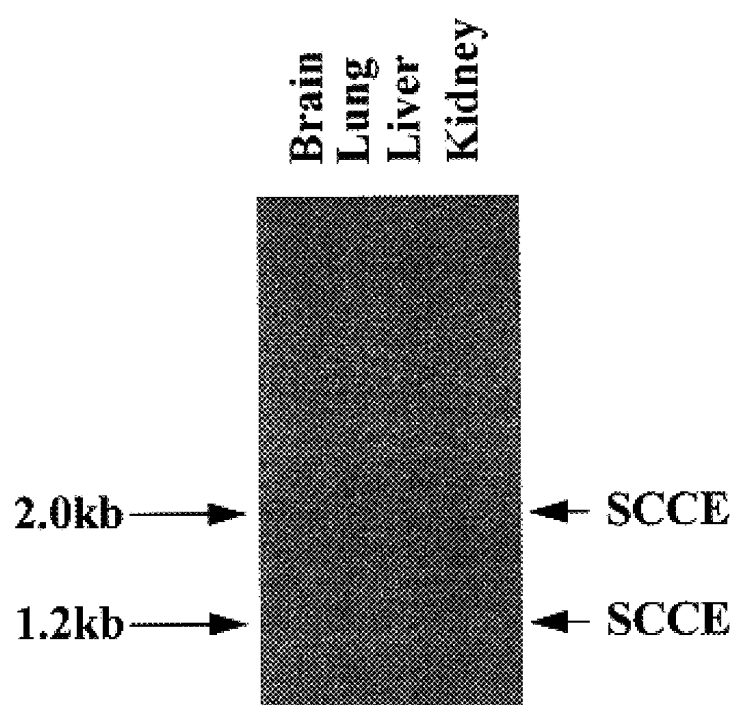


Fig. 12A

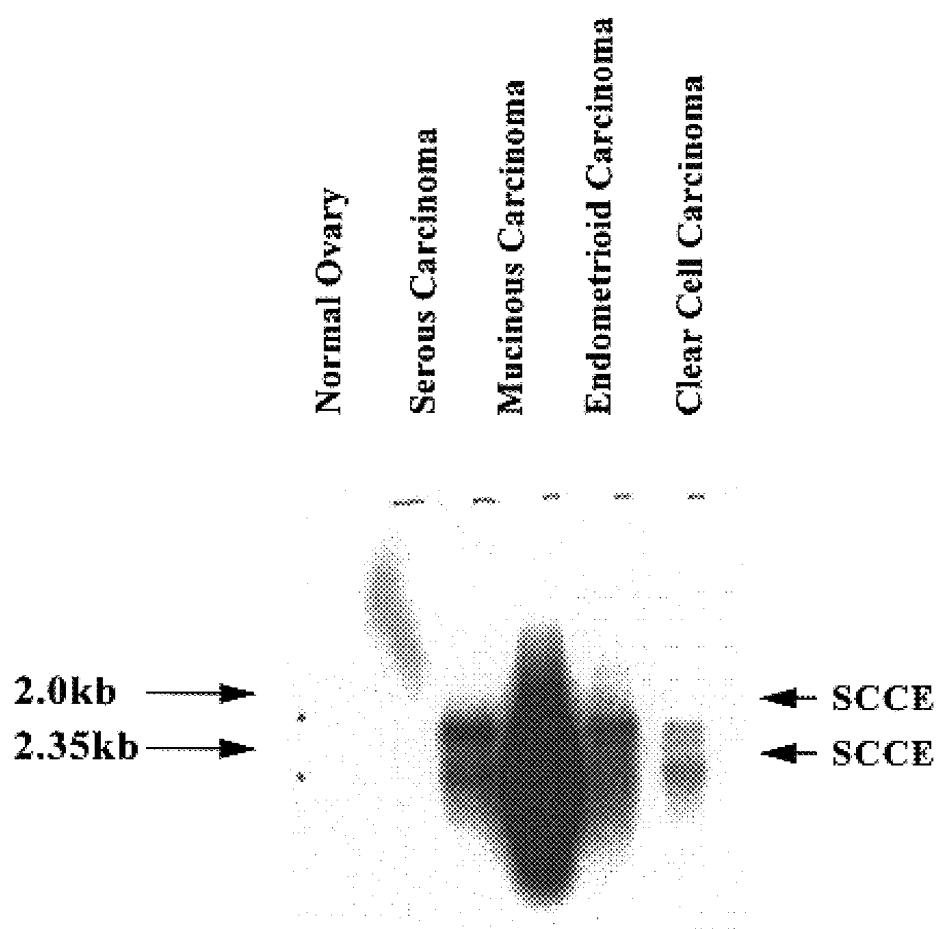


Fig. 12B

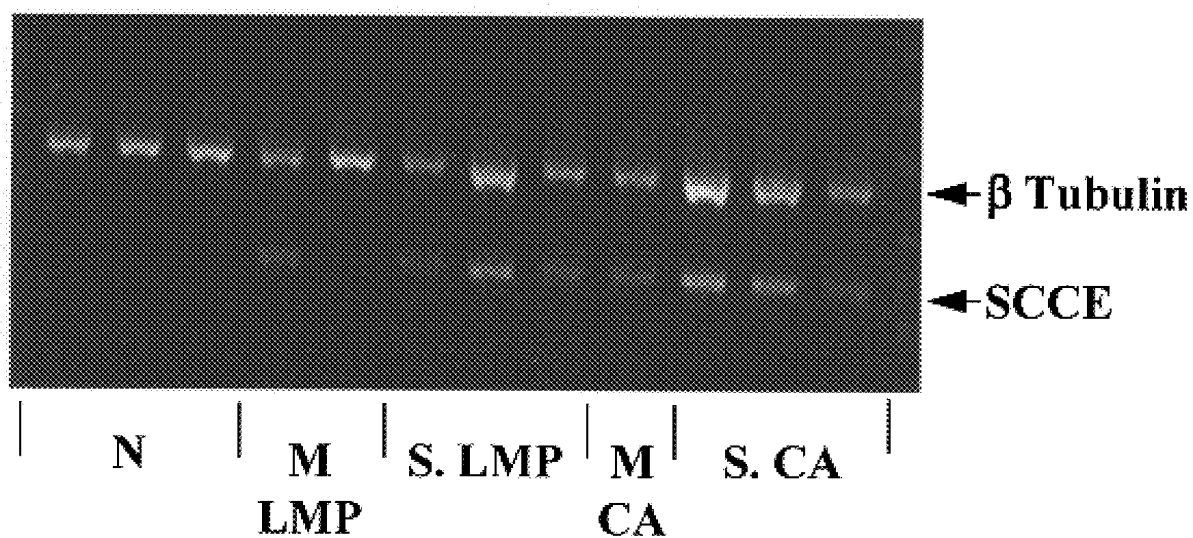


Fig. 13A

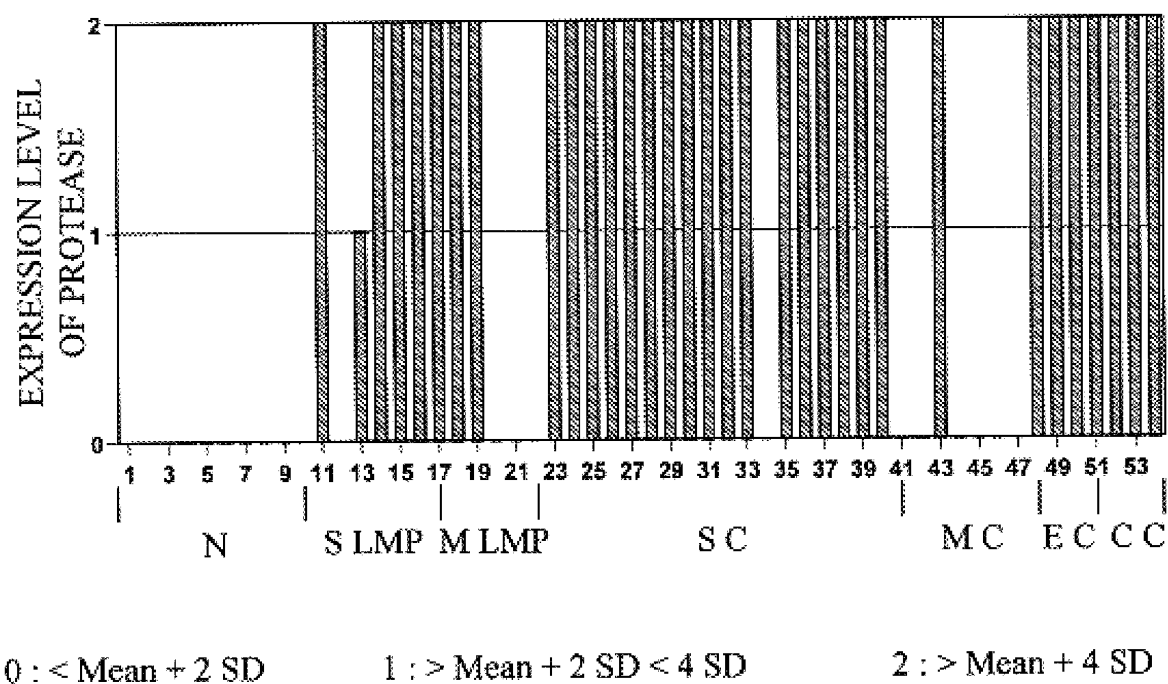


Fig. 13B

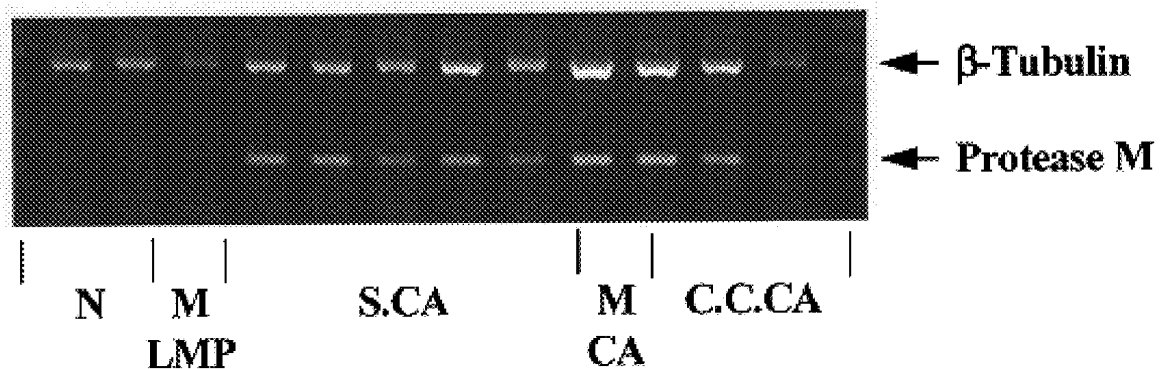


Fig. 14 **TADG12**

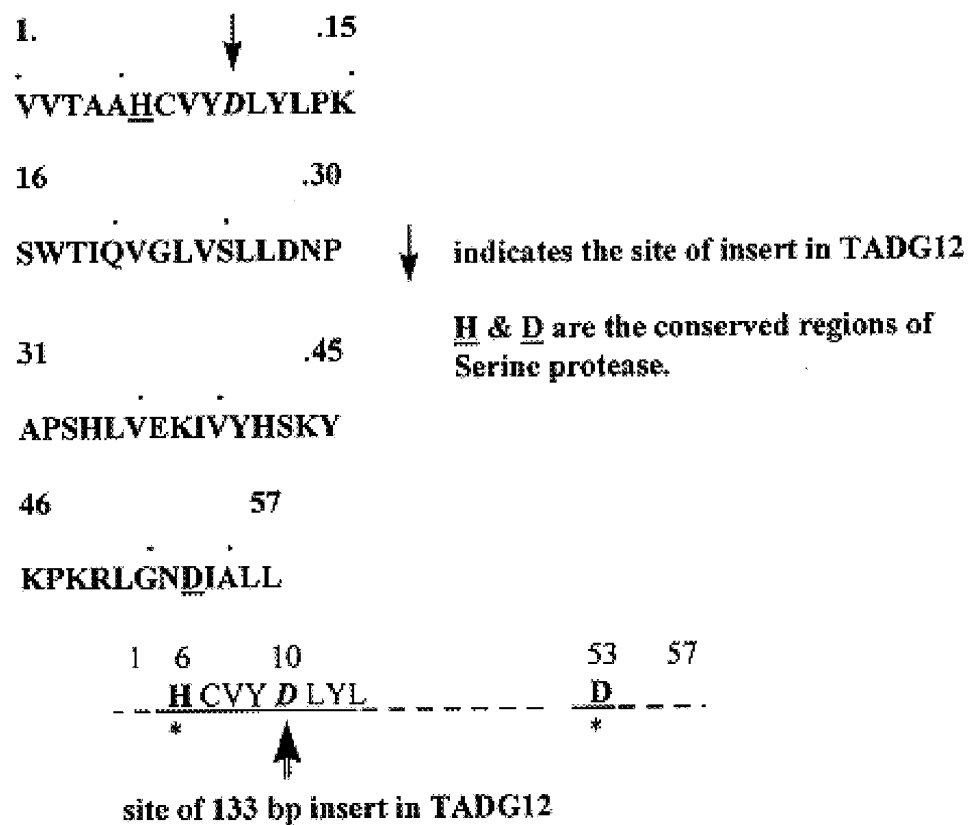


Fig. 15

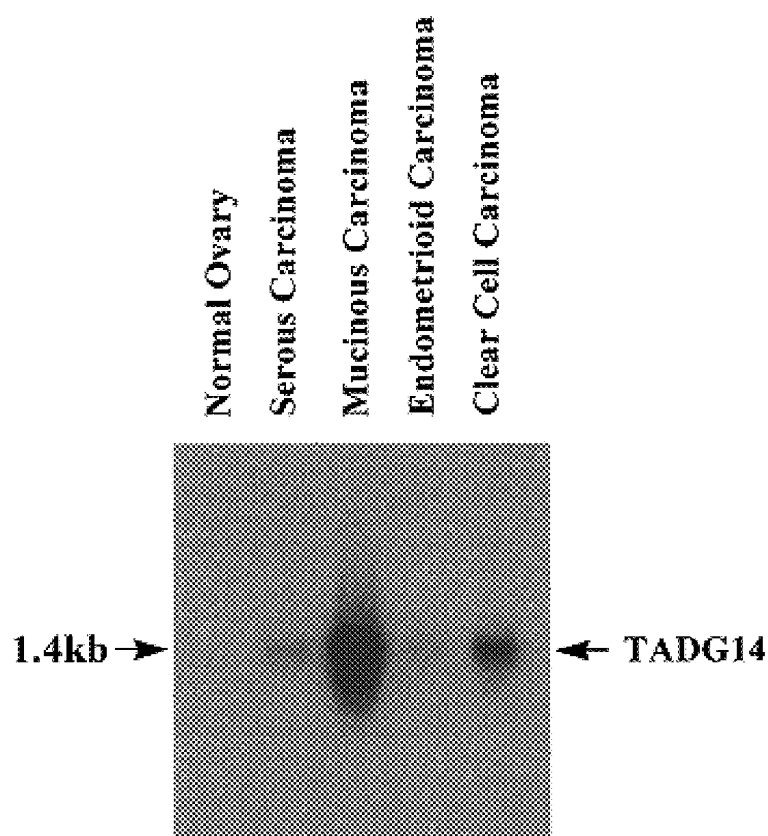


Fig. 16A

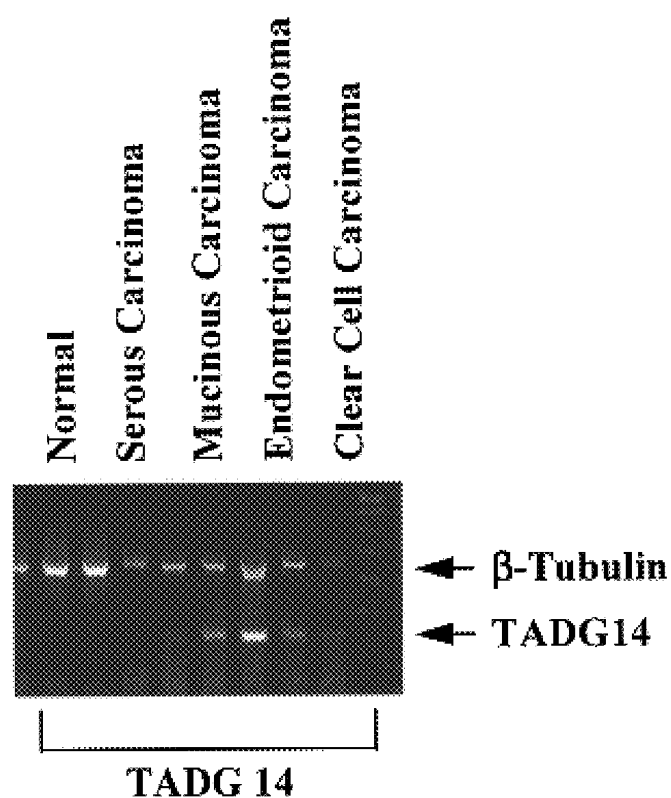


Fig. 16B

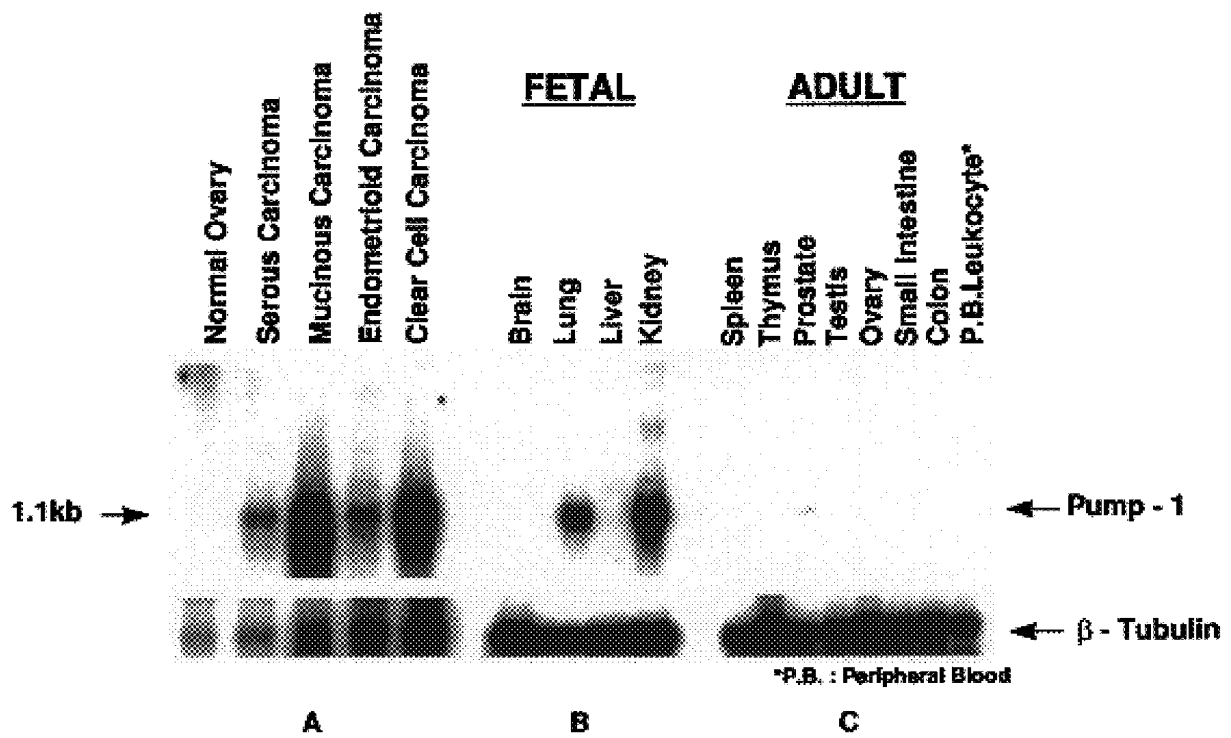


Fig. 17

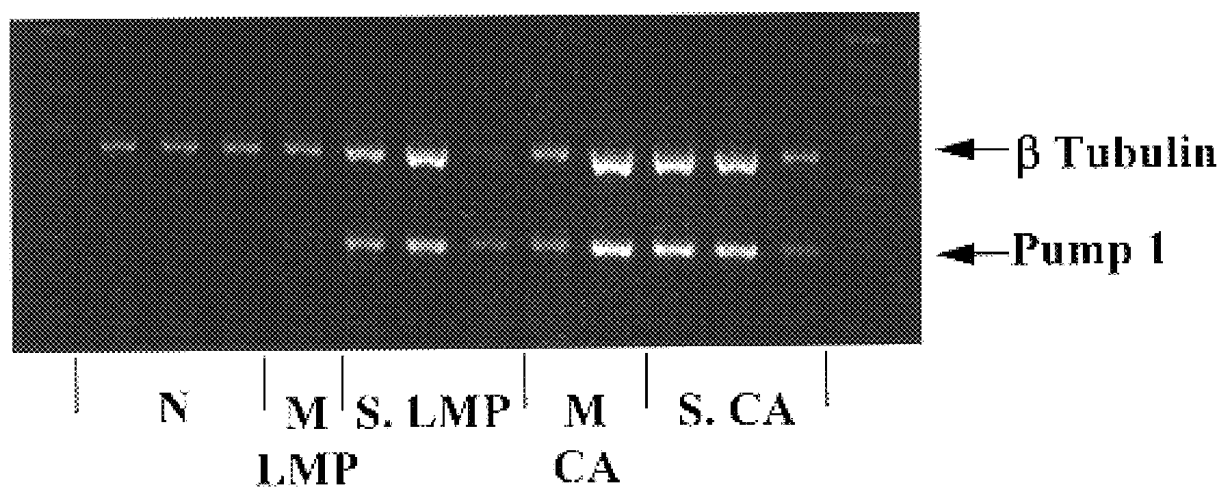


Fig. 18A

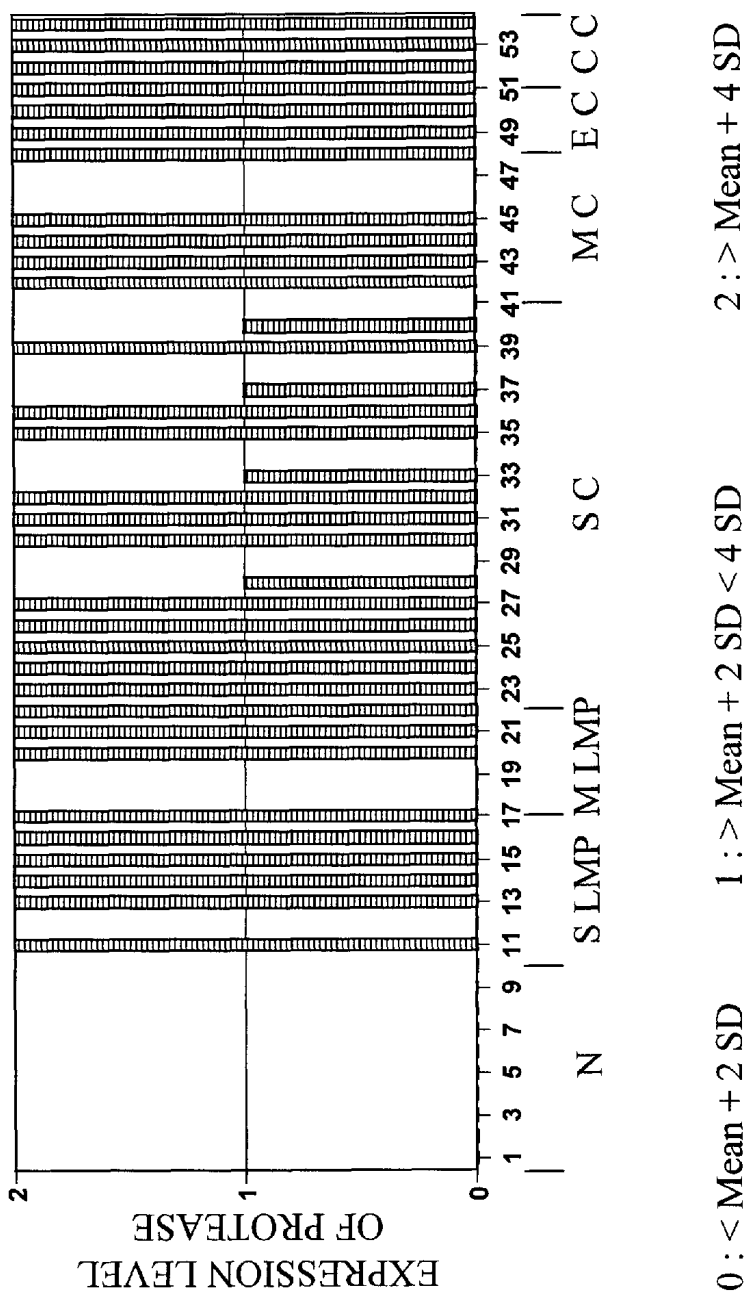


Fig. 18B

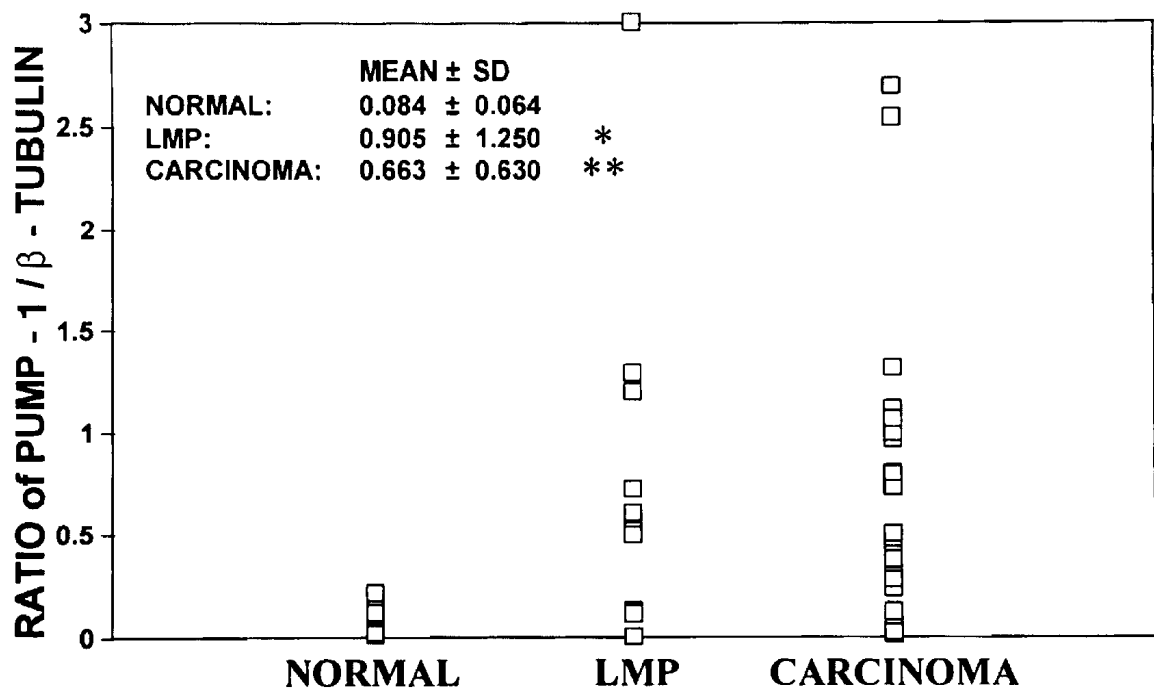


Fig. 19

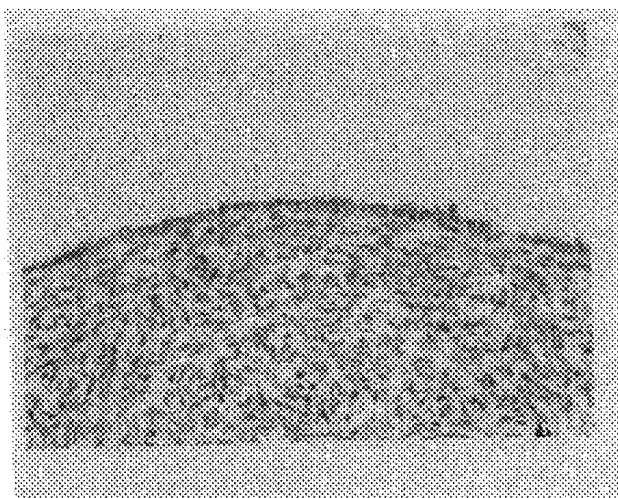


Fig. 20A

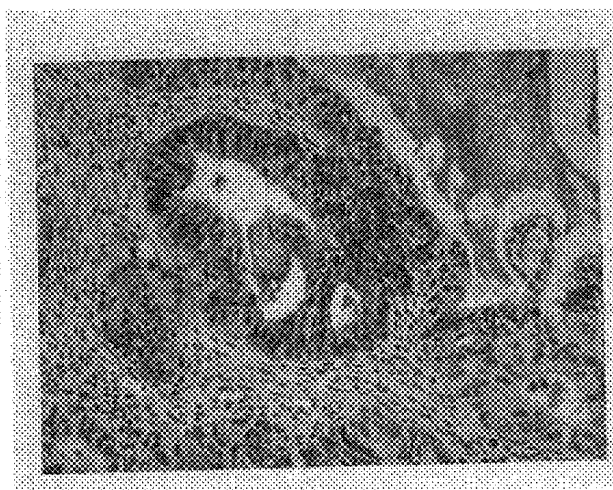


Fig. 20B

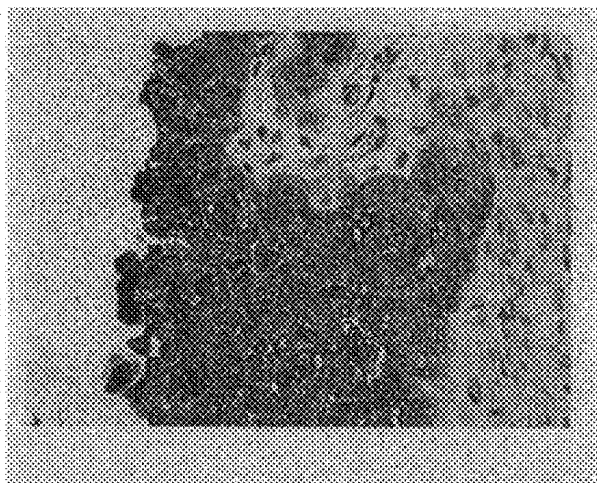


Fig. 20C

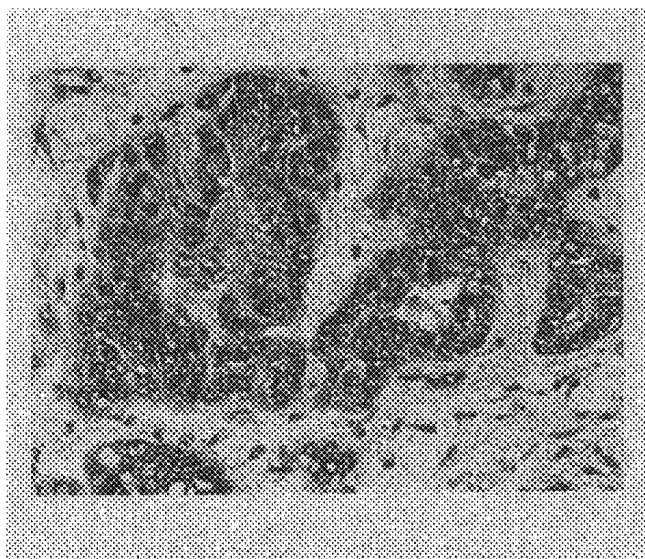


Fig. 20D

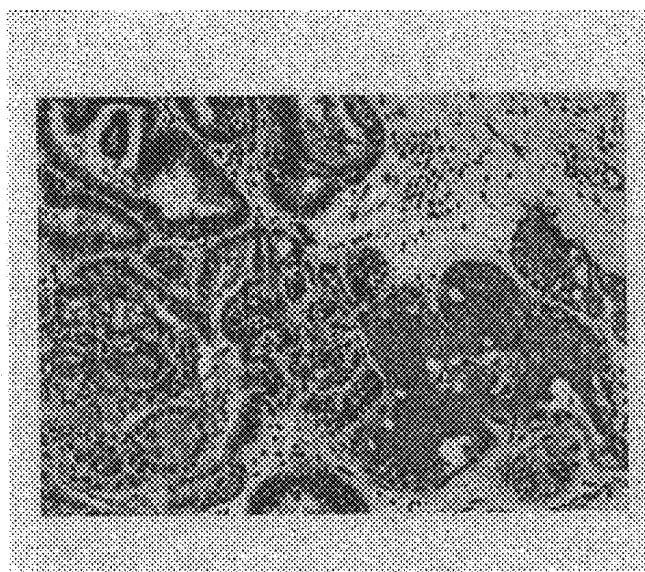


Fig. 20E

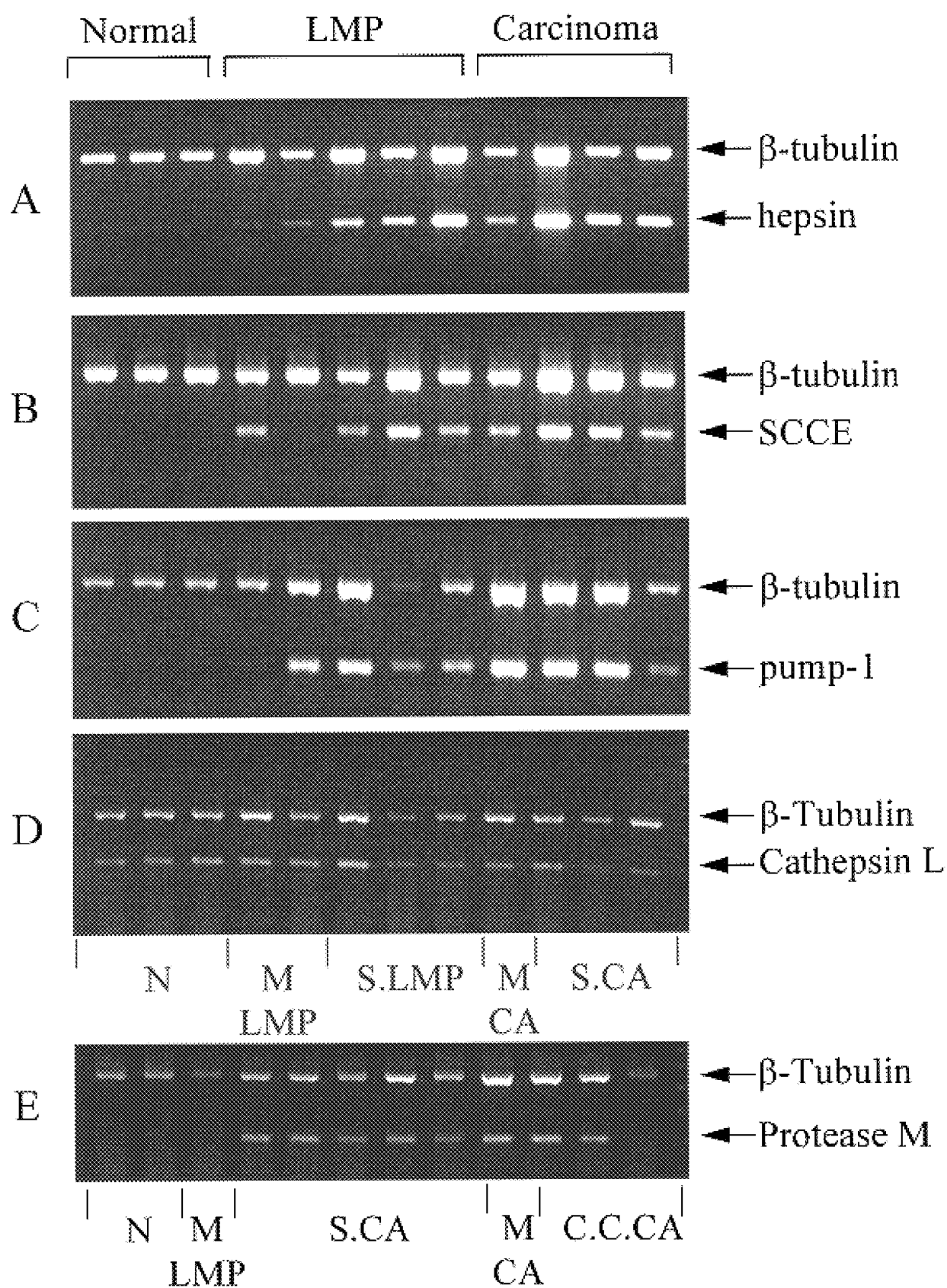


Fig. 21

METHODS FOR THE EARLY DIAGNOSIS OF OVARIAN, BREAST AND LUNG CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part application and claims benefit of priority under 35 U.S.C. 120 of U.S. Ser. No. 09/039,211, filed Mar. 14, 1998, now pending, which claims benefit of priority under 35 U.S.C. 119(e) of U.S. provisional application No. 60/041,404, filed Mar. 19, 1997, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

Generally, the present invention relates to the fields of molecular biology and medicine. More specifically, the present invention is in the field of ovarian and other cancer diagnosis.

2. Background of the Invention

To date, ovarian cancer remains the number one killer of women with gynecologic malignant hyperplasia. Approximately 75% of women diagnosed with such cancers are already at an advanced stage (III and IV) of the disease at their initial diagnosis. During the past 20 years, neither diagnosis nor five year survival rates have greatly improved for these patients. This is substantially due to the high percentage of high-stage initial detections of the disease. Therefore, the challenge remains to develop new markers that improve early diagnosis and thereby reduce the percentage of high-stage initial diagnoses.

Extracellular proteases have already been implicated in the growth, spread and metastatic progression of many cancers, due to the ability of malignant cells not only to grow in situ, but to dissociate from the primary tumor and to invade new surfaces. The ability to disengage from one tissue and re-engage the surface of another tissue is what provides for the morbidity and mortality associated with this disease. Therefore, extracellular proteases may be good candidates for markers of neoplastic development.

In order for malignant cells to grow, spread or metastasize, they must have the capacity to invade local host tissue, dissociate or shed from the primary tumor, and for metastasis to occur, enter and survive in the bloodstream, implant by invasion into the surface of the target organ and establish an environment conducive for new colony growth (including the induction of angiogenic and growth factors). During this progression, natural tissue barriers have to be degraded, including basement membranes and connective tissue. These barriers include collagen, laminin, proteoglycans and extracellular matrix glycoproteins, including fibronectin. Degradation of these natural barriers, both those surrounding the primary tumor and at the sites of metastatic invasion, is believed to be brought about by the action of a matrix of extracellular proteases.

Proteases have been classified into four families: serine proteases, metallo-proteases, aspartic proteases and cysteine proteases. Many proteases have been shown to be involved in the human disease process and these enzymes are targets for the development of inhibitors as new therapeutic agents. Additionally, certain individual proteases have been shown to be induced and overexpressed in a diverse group of cancers, and as such, are potential candidates for markers of early diagnosis and possible therapeutic intervention. A group of examples are shown in Table 1.

TABLE 1

Known proteases expressed in various cancers				
	Gastric	Brain	Breast	Ovarian
Serine Proteases:	uPA PAI-1	uPA PAI-1 tPA	NES-1 uPA	NES-1 uPA PAI-2
Cysteine Proteases:	Cathepsin B Cathepsin L	Cathepsin L	Cathepsin B Cathepsin L	Cathepsin B Cathepsin L
Metallo- proteases:	Matrilysin* Collagenase* Stromelysin-1*	Matrilysin Stromelysin Gelatinase B	Stromelysin-3 MMP-8 MMP-9 Gelatinase A	MMP-2

15 uPA, Urokinase-type plasminogen activator; tPA, Tissue-type plasminogen activator; PAI-I, Plasminogen activator 0 inhibitors; PAI-2, Plasminogen activator inhibitors; NES-1, Normal epithelial cell-specific-1; MMP, Matrix P metallo-protease. *Overexpressed in gastrointestinal ulcers.

Significantly, there is a good body of evidence supporting the downregulation or inhibition of individual proteases and the reduction in invasive capacity or malignancy. In work by Clark et al., inhibition of in vitro growth of human small cell lung cancer was demonstrated using a general serine protease inhibitor. More recently, Torres-Rosedo et al., [*Proc. Natl. Acad. Sci. USA*, 90, 7181-7185 (1993)] demonstrated an inhibition of hepatoma tumor cell growth using specific antisense inhibitors for the serine protease hepsin gene. Metastatic potential of melanoma cells has also been shown to be reduced in a mouse model using a synthetic inhibitor (batimastat) of metallo-proteases. Powell et al. [*Cancer Research*, 53, 417-422 (1993)] presented evidence to confirm that the expression of extracellular proteases in relatively non-invasive tumor cells enhances their malignant progression using a tumorigenic, but non-metastatic, prostate cell line. Specifically, enhanced metastasis was demonstrated after introducing and expressing the PUMP-1 metallo-protease gene. There is also a body of data to support the notion that expression of cell surface proteases on relatively non-metastatic cell types increases the invasive potential of such cells.

Thus, the prior art is deficient in a good tumor marker useful as an indicator of early disease, particularly for ovarian cancers. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

This invention allows for the detection of cancer, especially ovarian cancer, by screening for PUMP-1 mRNA in tissue, which is indicative of the PUMP-1 protease, which is shown herein to be specifically associated with the surface of 80 percent of ovarian and other tumors. Proteases are considered to be an integral part of tumor growth and metastasis, and therefore, markers indicative of their presence or absence are useful for the diagnosis of cancer. Furthermore, the present invention is useful for treatment (i.e., by inhibiting PUMP-1 or expression of PUMP-1), for targeted therapy, for vaccination, etc.

In one embodiment of the present invention, there is provided a method of diagnosing cancer in an individual, comprising the steps of (a) obtaining a biological sample from an individual; and (b) detecting PUMP-1 in the sample. Usually, the presence of PUMP-1 in the sample is indicative of the presence of cancer in the individual, and the absence of PUMP-1 in the sample is indicative of the absence of cancer in the individual.

In one embodiment of the present invention, there is provided a method for detecting malignant hyperplasia in a

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biological sample, comprising the steps of (a) isolating mRNA from the sample; and (b) detecting PUMP-1 mRNA in the sample. Typically, the presence of the PUMP-1 mRNA in the sample is indicative of the presence of malignant hyperplasia, and the absence of the PUMP-1 mRNA in the sample is indicative of the absence of malignant hyperplasia.

In one embodiment of the present invention, there is provided a method for detecting malignant hyperplasia in a biological sample, comprising the steps of (a) isolating protein from the sample; and (b) detecting PUMP-1 protein in the sample. Generally, the presence of the PUMP-1 protein in the sample is indicative of the presence of malignant hyperplasia, and the absence of the PUMP-1 protein in the sample is indicative of the absence of malignant hyperplasia.

In one embodiment of the present invention, there is provided a method of inhibiting expression of endogenous PUMP-1 in a cell, comprising the step of (a) introducing a vector into a cell, wherein the vector comprises a PUMP-1 gene in opposite orientation operably linked to elements necessary for expression. Upon expression of the vector, the cell produces PUMP-1 antisense mRNA, which hybridizes to endogenous PUMP-1 mRNA, thereby inhibiting expression of endogenous PUMP-1 in the cell.

In one embodiment of the present invention, there is provided a method of inhibiting PUMP-1 protein in a cell, comprising the step of (a) introducing an antibody into a cell which is specific for a PUMP-1 protein or a fragment thereof. Generally, binding of the antibody to the PUMP-1 protein inhibits the PUMP-1 protein.

In one embodiment of the present invention, there is provided a method of targeted therapy to an individual, comprising the step of (a) administering a compound to an individual, wherein the compound has a targeting moiety and a therapeutic moiety. Specifically, the targeting moiety is specific for PUMP-1.

In one embodiment of the present invention, there is provided a method of vaccinating an individual against PUMP-1, comprising the step of (a) inoculating an individual with a PUMP-1 protein or fragment thereof which lacks PUMP-1 protease activity. Typically, inoculation with the PUMP-1 protein or fragment thereof elicits an immune response in the individual, thereby vaccinating the individual against PUMP-1.

In one embodiment of the present invention, there is provided a method of producing immune-activated cells directed toward PUMP-1, comprising the steps of exposing dendritic cells to a PUMP-1 protein or fragment thereof which lacks PUMP-1 protease activity. Usually, exposure to the PUMP-1 protein or fragment thereof activates the dendritic cells, thereby producing immune-activated cells directed toward PUMP-1.

In one embodiment of the present invention, there is provided an immunogenic composition, comprising an immunogenic fragment of a PUMP-1 protein and an appropriate adjuvant.

In one embodiment of the present invention, there is provided an oligonucleotide having a sequence complementary to SEQ ID No. 29, as well as a composition comprising the oligonucleotide and a physiologically acceptable carrier. Additionally, there is provided a method of treating a neoplastic state in an individual in need of such treatment, comprising the step of (a) administering to the individual an effective dose of the above-described oligonucleotide.

In one embodiment of the present invention, there is provided a method of screening for compounds that inhibit

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PUMP-1 activity, comprising the steps of (a) contacting a sample with a compound, wherein the sample comprises PUMP-1 protein; and (b) assaying for PUMP-1 protease activity. Typically, a decrease in PUMP-1 protease activity in the presence of the compound relative to PUMP-1 protease activity in the absence of the compound is indicative of a compound that inhibits PUMP-1 activity.

In one embodiment of the present invention, there is provided a method for detecting ovarian malignant hyperplasia in a biological sample, comprising the steps of (a) isolating the proteases or protease mRNA present in the biological sample; and (b) detecting specific proteases or protease mRNA present in the biological sample. Representative proteases are hepsin, protease M, complement factor B, SCCE, other serine proteases indicated in lanes 2 and 4 of FIG. 1, cathepsin L and PUMP-1.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention. These embodiments are given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

The appended drawings have been included herein so that the above-recited features, advantages and objects of the invention will become clear and can be understood in detail. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and should not be considered to limit the scope of the invention.

FIG. 1 shows agarose gel comparison of PCR products derived from normal and carcinoma cDNA.

FIG. 2 shows Northern blot analysis of ovarian tumors using hepsin, SCCE, PUMP-1, TADG-14 and β -tubulin probes.

FIG. 3 shows amplification with serine protease redundant primers: histidine sense (S1) with aspartic acid antisense (AS1), using normal cDNA (Lane 1) and tumor cDNA (Lane 2); and histidine sense (S1) with serine antisense (AS2), using normal cDNA (Lane 3) and tumor cDNA (Lane 4).

FIG. 4 shows amplification with cysteine protease redundant primers. Normal (Lane 1), low malignant potential (Lane 2), serious carcinoma (Lane 3), mucinous carcinoma (Lane 4), and clear cell carcinoma (Lane 5).

FIG. 5 shows amplification with metallo-protease redundant primers. Normal (Lane 1), low malignant potential (Lane 2), serious carcinoma (Lane 3), mucinous carcinoma (Lane 4), and clear cell carcinoma (Lane 5).

FIG. 6 shows amplification with specific primers directed towards the serine protease, hepsin. Expression in normal (Lanes 1-3), low malignant potential tumors (Lanes 4-8), and ovarian carcinomas (Lanes 9-12).

FIG. 7 shows hepsin expression levels in normal, low malignant potential tumors, and ovarian carcinomas. S=serious, M=mucinous, LMP=low malignant potential.

FIG. 8 shows serine protease stratum corneum chymotrypsin enzyme (SCCE) expression in normal, low malignant potential tumors, and ovarian carcinomas.

FIG. 9 shows metallo-protease PUMP-1 (MMP-7) gene expression in normal (lanes 1-2) and ovarian carcinomas tissue (Lanes 3-10).

FIG. 10 shows Northern blot analysis of hepsin expression in normal ovary and ovarian carcinomas. (FIG. 10A), lane 1, normal ovary (case 10); lane 2, serous carcinoma

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(case 35); lane 3, mucinous carcinoma (case 48); lane 4, endometrioid carcinoma (case 51); and lane 5, clear cell carcinoma (case 54). In cases 35, 51 and 54, more than a 10-fold increase in the hepsin 1.8 kb transcript abundance was observed. Northern blot analysis of hepsin in normal human fetal (FIG. 10B) and adult tissues (FIG. 10C). Significant overexpression of the hepsin transcript is noted in both fetal liver and fetal kidney. Notably, hepsin overexpression is not observed in normal adult tissue. Slight expression above the background level is observed in the adult prostate.

FIG. 11 shows hepsin expression in normal (N), mucinous (M) and serous (S) low malignant potential (LMP) tumors and carcinomas (CA). FIG. 11A shows quantitative PCR of hepsin and internal control β -tubulin. FIG. 11B shows a bar graph of expression of hepsin in 10 normal ovaries and 44 ovarian carcinoma samples.

FIG. 12 shows northern blot analysis of mRNA expression of the SCCE gene in fetal tissue (FIG. 12A) and in ovarian tissue (FIG. 12B).

FIG. 13A shows a comparison of quantitative PCR of cDNA from normal ovary and ovarian carcinomas. FIG. 13B shows a bar graph comparing the ratio of SCCE to β -tubulin in 10 normal and 44 ovarian carcinoma tissues.

FIG. 14 shows a comparison by quantitative PCR of normal and ovarian carcinoma expression of mRNA for protease M.

FIG. 15 shows the TADG-12 catalytic domain including an insert near the His 5'-end.

FIG. 16 shows northern blot analysis comparing TADG-14 expression in normal and ovarian carcinoma tissues (FIG. 16A), and preliminary quantitative PCR amplification of normal and carcinoma cDNAs using specific primers for TADG-14 (FIG. 16B).

FIG. 17A shows Northern blot analysis of PUMP-1 mRNA from normal ovary and ovarian carcinomas. Lane 1, normal ovary; lane 2, serous carcinoma; lane 3, mucinous carcinoma; lane 4, endometrioid carcinoma; lane 5, clear cell carcinoma. PUMP-1 transcripts are detected only in carcinoma cases (lanes 2-5). FIG. 17B shows that among normal human fetal tissues, fetal lung and fetal kidney show increased transcript. FIG. 17C shows that PUMP-1 overexpression is not observed in normal human adult tissues. Slight expression above the background level is observed in the prostate.

FIG. 18A shows quantitative PCR analysis of PUMP-1 expression. Cases 3, 4 and 9 are normal ovaries. Cases 19,

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21, 14, 15 and 16 are LMP tumors. Cases 43, 23, 36 and 37 are ovarian carcinomas. Expression levels of PUMP-1 relative to β -tubulin are significantly elevated in 8 or 9 tumor cases compared to that of normal ovaries. FIG. 18B shows the ratio of mRNA expression of PUMP-1 compared to the internal control β -tubulin in 10 normal and 44 ovarian carcinomas.

FIG. 19 shows the ratio of PUMP-1 expression to expression of β -tubulin in normal ovary, LMP tumors and ovarian carcinomas. PUMP-1 mRNA expression levels were significantly elevated in LMP tumor ($p < 0.05$) and carcinoma ($p < 0.0001$) compared to that in normal ovary. All 10 samples of individual normal ovary showed low levels of PUMP-1 expression.

FIG. 20 shows immunohistochemical staining of PUMP-1 in normal ovary and ovarian tumor tissues. FIG. 20A shows normal ovarian epithelium shows no PUMP-1 immunoreactivity ($\times 20$). FIG. 20B shows intense staining of secretory vessels in mucinous tumors ($\times 20$). FIG. 20C shows cytoplasmic staining of PUMP-1 in serous tumors ($\times 20$). FIG. 20D shows clear cell tumors ($\times 100$). FIG. 20E shows secretion of PUMP-1 to the extracellular environment of endometrioid tumors ($\times 100$).

FIG. 21 shows a comparison of PCR amplified products for the hepsin, SCCE, protease M, PUMP-1 and Cathepsin L genes.

DETAILED DESCRIPTION OF THE INVENTION

This invention identifies a PUMP-1 protease on ovarian and other tumor cells which is characteristic of this type of cancer, and in various combinations with other proteases, is characteristic of individual tumor types. Such information can provide the basis for diagnostic tests (assays or immunohistochemistry), prognostic evaluation (depending on the display pattern) and therapeutic intervention utilizing either antibodies directed at the protease, antisense vehicles for downregulation or protease inhibitors both from established inhibition data and/or for the design of new drugs. Long-term treatment of tumor growth, invasion and metastasis has not succeeded with existing chemotherapeutic agents—most tumors become resistant to drugs after multiple cycles of chemotherapy. The full-length sequence of PUMP-1 (SEQ ID No. 29) is as follows:

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1  AAGAACAATT GTCTCTGGAC GGCAGCTATG CGACTCACCG TGCTGTGTGC
51  TGTGTGCCTG CTGCCTGGCA GCCTGGCCCT GCCGCTGCCT CAGGAGGCGG
101 GAGGCATGAG TGAGCTACAG TGGGAACAGG CTCAGGACTA TCTCAAGAGA
151 TTTTATCTCT ATGACTCAGA AACAAAAAAT GCCAACAGTT TAGAAGCCAA
201 ACTCAAGGAG ATGCAAAAAT TCTTTGGCCT ACCTATAACT GGAATGTTAA
251 ACTCCCGCGT CATAGAAATA ATGCAGAAGC CCAGATGTGG AGTGCCAGAT
301 GTTGCAGAAT ACTCACTATT TCCAAATAGC CCAAATGGA CTTCCAAAGT
351 GGTCACCTAC AGGATCGTAT CATATACTCG AGACTTACCG CATATTACAG
401 TGGATCGATT AGTGTCAAAG GCTTTAAACA TGTGGGGCAA AGAGATCCCC

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-continued

451 CTGCATTTC A GGAAAGTTGT ATGGGGAAC T GCTGACATCA TGATTGGCTT
 501 TGC GCGAGGA GCTCATGGGG ACTCCTACCC ATTTGATGGG CCAGGAAACA
 551 CGCTGGCTCA TGCCTTTGCG CCTGGGACAG GTCTCGGAGG AGATGCTCAC
 601 TTCGATGAGG ATGAACGCTG GACGGATGGT AGCAGTCTAG GGATTAACTT
 651 CCTGTATGCT GCAACTCATG AACTTGGCCA TTCTTTGGGT ATGGGACATT
 701 CCTCTGATCC TAATGCAGTG ATGTATCCAA CCTATGGAAA TGGAGATCCC
 751 CAAAATTTTA AACTTTCCCA GGATGATATT AAAGGCATTC AGAAACTATA
 801 TGGAAAGAGA AGTAATTC A GAAAGAAATA GAAACTTCAG GCAGAACATC
 851 CATTCAATTC TTCATTGGAT TGTATATCAT TGTGACACA TCAGAATTGA
 901 TAAGCACTGT TCCTCCACTC CATTTAGCAA TTATGTCACC CTTTTTTATT
 951 GCAGTTGGTT TTTGAATGTC TTTCACCTCT TTTATTGGTT AAACCTCTTT
 1001 ATGGTGTGAC TGTGTCTTAT TCCATCTATG AGCTTTGTCA GTGCGCGTAG
 1051 ATGTCAATAA ATGTTACATA CACAAATA

The present invention is directed toward a method of diagnosing cancer in an individual, comprising the steps of (a) obtaining a biological sample from an individual; and (b) detecting PUMP-1 in the sample. Generally, the presence of PUMP-1 in the sample is indicative of the presence of cancer in the individual, and the absence of PUMP-1 in the sample is indicative of the absence of cancer in the individual.

The present invention is directed toward a method for detecting malignant hyperplasia in a biological sample, comprising the steps of (a) isolating mRNA from the sample; and (b) detecting PUMP-1 mRNA in the sample. Typically, the presence of the PUMP-1 mRNA in the sample is indicative of the presence of malignant hyperplasia, and the absence of the PUMP-1 mRNA in the sample is indicative of the absence of malignant hyperplasia. This method may further comprise the step(s) of comparing the PUMP-1 mRNA to reference information, wherein the comparison provides a diagnosis of the malignant hyperplasia; and/or comparing the PUMP-1 mRNA to reference information, wherein the comparison determines a treatment of the malignant hyperplasia. Preferably, detection of the PUMP-1 mRNA is by PCR amplification, and even more preferably, using primers SEQ ID No. 8 and SEQ ID No. 9.

The present invention is directed toward a method for detecting malignant hyperplasia in a biological sample, comprising the steps of (a) isolating protein from the sample; and (b) detecting PUMP-1 protein in the sample. Typically, the presence of the PUMP-1 protein in the sample is indicative of the presence of malignant hyperplasia, and the absence of the PUMP-1 protein in the sample is indicative of the absence of malignant hyperplasia. This method may further comprise the step(s) of comparing the PUMP-1 protein to reference information, wherein the comparison provides a diagnosis of the malignant hyperplasia; and/or comparing the PUMP-1 protein to reference information, wherein the comparison determines a treatment of the malignant hyperplasia. Typically, detection is by immunoaffinity to an antibody, wherein the antibody is specific for PUMP-1.

The present invention is directed toward a method of inhibiting expression of endogenous PUMP-1 in a cell, comprising the step of (a) introducing a vector into a cell, wherein the vector comprises a PUMP-1 gene in opposite orientation operably linked to elements necessary for

expression. Upon expression of the vector in the cell, PUMP-1 antisense mRNA is produced, which hybridizes to endogenous PUMP-1 mRNA, thereby inhibiting expression of endogenous PUMP-1 in the cell.

The present invention is directed toward a method of inhibiting PUMP-1 protein in a cell, comprising the step of (a) introducing an antibody into a cell, which is specific for a PUMP-1 protein or a fragment thereof, whereupon binding of the antibody to the PUMP-1 protein inhibits the PUMP-1 protein.

The present invention is directed toward a method of targeted therapy to an individual, comprising the step of (a) administering a compound to an individual, wherein the compound has a targeting moiety and a therapeutic moiety. In the present invention, the targeting moiety is specific for PUMP-1. Preferably, the targeting moiety is an antibody specific for PUMP-1 or a ligand or ligand binding domain that binds PUMP-1. Likewise, the therapeutic moiety is preferably a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant or a cytotoxic agent. Generally, the individual suffers from a disease such as ovarian cancer, lung cancer, prostate cancer, colon cancer or another cancer in which PUMP-1 is overexpressed.

The present invention is directed toward a method of vaccinating an individual against PUMP-1 comprising the step of (a) inoculating an individual with a PUMP-1 protein or fragment thereof, which lacks PUMP-1 protease activity. Generally, inoculation with the PUMP-1 protein or fragment thereof elicits an immune response in the individual, thereby vaccinating the individual against PUMP-1. Typically, the individual has cancer, is suspected of having cancer or is at risk of getting cancer. Preferably, the PUMP-1 fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 30, 31, 32, 33, 50, 70, 110, 111, 150, 151 or 152.

The present invention is directed toward a method of producing immune-activated cells directed toward PUMP-1, comprising the steps of exposing dendritic cells to a PUMP-1 protein or fragment thereof, which lacks PUMP-1 protease activity. Typically, exposure to the PUMP-1 protein or fragment thereof activates the dendritic cells, thereby producing immune-activated cells directed toward PUMP-1. Generally, the immune-activated cells are B-cells, T-cells

and/or dendrites. Preferably, the PUMP-1 fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 30, 31, 32, 33, 50, 70, 110, 111, 150, 151 or 152. Oftentimes, the dendritic cells are isolated from an individual prior to exposure and then reintroduced into the individual subsequent to the exposure. Typically, the individual has cancer, is suspected of having cancer or is at risk of getting cancer.

The present invention is directed toward an immunogenic composition, comprising an immunogenic fragment of a PUMP-1 protein and an appropriate adjuvant. Preferably, the fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 30, 31, 32, 33, 50, 70, 110, 111, 150, 151 or 152.

The present invention is directed toward an oligonucleotide having a sequence complementary to SEQ ID No. 29. The present invention further provides a composition comprising the above-described oligonucleotide and a physiologically acceptable carrier. The present invention is directed toward a method of treating a neoplastic state in an individual in need of such treatment, comprising the step of (a) administering to the individual an effective dose of the above-described oligonucleotide. Typically, the neoplastic state is ovarian cancer, breast cancer, lung cancer, colon cancer, prostate cancer or another cancer in which PUMP-1 is overexpressed.

The present invention is directed toward a method of screening for compounds that inhibit PUMP-1 activity, comprising the steps of (a) contacting a sample with a compound, wherein the sample comprises PUMP-1 protein; and (b) assaying for PUMP-1 protease activity. Usually, a decrease in the PUMP-1 protease activity in the presence of the compound relative to PUMP-1 protease activity in the absence of the compound is indicative of a compound that inhibits PUMP-1 activity.

The present invention is directed toward a method for detecting ovarian malignant hyperplasia in a biological sample, comprising the steps of (a) isolating the proteases or protease mRNA present in the biological sample; and (b) detecting specific proteases or protease mRNA present in the biological sample. Representative proteases are hepsin, protease M, complement factor B, SCCE, other serine proteases indicated in lanes 2 and 4 of FIG. 1, cathepsin L and PUMP-1. This method may further comprise the step of comparing the specific proteases or protease mRNA detected to reference information, wherein the comparison provides a diagnoses of the malignant hyperplasia; and/or comparing the specific proteases or protease mRNA detected to reference information, wherein the comparison determines a treatment of the malignant hyperplasia. Generally, the protease mRNA is detected by amplification of total mRNA, or the protease is detected with an antibody.

According to the above descriptions of the present invention, representative biological samples are blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells. Typically, detection of the PUMP-1 is by means such as Northern blot, Western blot, PCR, dot blot, ELISA sandwich assay, radioimmunoassay, DNA array chips and flow cytometry. Generally, the cancer is ovarian, breast, lung, colon, prostate or others in which PUMP-1 is overexpressed.

An additional object of the present invention is a number of nucleic acid sequences that are useful in its practice. These nucleic acid sequences are listed in Table 2. It is anticipated that these nucleic acid sequences be used in

mixtures to accomplish the utility of this invention. Features of such mixtures include: SEQ ID Nos. 1 & 2; SEQ ID Nos. 1 & 3; SEQ ID Nos. 4 & 5; SEQ ID Nos. 6 & 7; and SEQ ID Nos. 8 & 9. The skilled artisan may be able to develop other nucleic acid sequences and mixtures thereof to accomplish the benefit of this invention, but it is advantageous to have the sequences listed in Table 2 available without undue experimentation.

It will be apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Maniatis, Fritsch & Sambrook, "Molecular Cloning: A Laboratory Manual" (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D. N. Glover ed. 1985); "Oligonucleotide Synthesis" (M. J. Gait ed. 1984); "Nucleic Acid Hybridization" (B. D. Hames & S. J. Higgins eds. 1985); "Transcription and Translation" (B. D. Hames & S. J. Higgins eds. 1984); "Animal Cell Culture" (R. I. Freshney, ed. 1986); "Immobilized Cells And Enzymes" (IRL Press, 1986); B. Perbal, "A Practical Guide To Molecular Cloning" (1984). Therefore, if appearing herein, the following terms shall have the definitions set out below.

As used herein, the term "cDNA" shall refer to the DNA copy of the mRNA transcript of a gene.

As used herein, the term "derived amino acid sequence" shall mean the amino acid sequence determined by reading the triplet sequence of nucleotide bases in the cDNA.

As used herein the term "screening a library" shall refer to the process of using a labeled probe to check whether, under the appropriate conditions, there is a sequence complementary to the probe present in a particular DNA library. In addition, "screening a library" could be performed by PCR.

As used herein, the term "PCR" refers to the polymerase chain reaction that is the subject of U.S. Pat. Nos. 4,683,195 and 4,683,202 to Mullis, as well as other improvements now known in the art.

The amino acid described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property of immunoglobulin-binding is retained by the polypeptide. NH₂ refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J Biol. Chem.*, 243:3552-59 (1969), abbreviations for amino acid residues may be used.

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues. The above Table is presented to correlate the three-letter and one-letter notations which may appear alternately herein.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication in vivo; i.e., capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so

as to bring about the replication of the attached segment. A "vector" may further be defined as a replicable nucleic acid construct, e.g., a plasmid or viral nucleic acid.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single-stranded form or as a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. The structure is discussed herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

An expression vector is a replicable construct in which a nucleic acid sequence encoding a polypeptide is operably linked to suitable control sequences capable of effecting expression of the polypeptide in a cell. The need for such control sequences will vary depending upon the cell selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter and/or enhancer, suitable mRNA ribosomal binding sites and sequences which control the termination of transcription and translation. Methods which are well known to those skilled in the art can be used to construct expression vectors containing appropriate transcriptional and translational control signals. See, for example, techniques described in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual* (2nd Ed.), Cold Spring Harbor Press, N.Y. A gene and its transcription control sequences are defined as being "operably linked" if the transcription control sequences effectively control transcription of the gene. Vectors of the invention include, but are not limited to, plasmid vectors and viral vectors. Preferred viral vectors of the invention are those derived from retroviruses, adenovirus, adeno-associated virus, SV40 virus, or herpes viruses. In general, expression vectors contain promoter sequences which facilitate the efficient transcription of the inserted DNA fragment and are used in connection with a specific host. The expression vector typically contains an origin of replication, promoter(s), terminator(s), as well as specific genes which are capable of providing phenotypic selection in transformed cells. The transformed hosts can be fermented and cultured according to means known in the art to achieve optimal cell growth.

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are typically determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating

transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters typically contain Shine-Dalgarno ribosome-binding sequences in addition to the -10 and -35 consensus sequences.

An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

A "signal sequence" can be included near the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90% or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., supra; DNA Cloning, Vols. I & II, supra; Nucleic Acid Hybridization, supra.

A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mam-

malian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others. A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate. Proteins can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{125}I , ^{131}I , and ^{186}Re . Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, β -glucuronidase, β -D-glucosidase, β -D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Pat. Nos. 3,654,090, 3,850,752, and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

A particular assay system developed and utilized in the art is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantity of both the label after which binding studies are conducted to determine the extent to which the labeled material binds to the cell receptors. In this way, differences in affinity between materials can be ascertained.

An assay useful in the art is known as a "cis/trans" assay. Briefly, this assay employs two genetic constructs, one of which is typically a plasmid that continually expresses a particular receptor of interest when transfected into an appropriate cell line, and the second of which is a plasmid that expresses a reporter such as luciferase, under the control of a receptor/ligand complex. Thus, for example, if it is desired to evaluate a compound as a ligand for a particular receptor, one of the plasmids would be a construct that results in expression of the receptor in the chosen cell line, while the second plasmid would possess a promoter linked to the luciferase gene in which the response element to the particular receptor is inserted. If the compound under test is an agonist for the receptor, the ligand will complex with the receptor, and the resulting complex will bind the response element and initiate transcription of the luciferase gene. The resulting chemiluminescence is then measured photometrically, and dose response curves are obtained and compared to those of known ligands. The foregoing protocol is described in detail in U.S. Pat. No. 4,981,784.

As used herein, the term "host" is meant to include not only prokaryotes but also eukaryotes such as yeast, plant and animal cells. A recombinant DNA molecule or gene which encodes a human PUMP-1 protein of the present invention

can be used to transform a host using any of the techniques commonly known to those of ordinary skill in the art. Especially preferred is the use of a vector containing coding sequences for the gene which encodes a human PUMP-1 protein of the present invention for purposes of prokaryote transformation. Prokaryotic hosts may include *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. Eukaryotic hosts include yeasts such as *Pichia pastoris*, mammalian cells and insect cells.

As used herein, "substantially pure DNA" means DNA that is not part of a milieu in which the DNA naturally occurs, by virtue of separation (partial or total purification) of some or all of the molecules of that milieu, or by virtue of alteration of sequences that flank the claimed DNA. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into a n autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by polymerase chain reaction (PCR) or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence, e.g., a fusion protein. Also included is a recombinant DNA which includes a portion of the nucleotides listed in SEQ ID No. 29 and which encodes an alternative splice variant of PUMP-1.

By a "substantially pure protein" is meant a protein which has been separated from at least some of those components which naturally accompany it. Typically, the protein is substantially pure when it is at least 60% (by weight) free from the proteins and other naturally-occurring organic molecules with which it is naturally associated in vivo. Preferably, the purity of the preparation (by weight) is at least 75%, more preferably at least 90%, and most preferably at least 99%. A substantially pure PUMP-1 protein may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a PUMP-1 polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography, such as immunoaffinity chromatography using an antibody specific for PUMP-1, polyacrylamide gel electrophoresis, or HPLC analysis. A protein is substantially free of naturally associated components when it is separated from at least some of those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be, by definition, substantially free from its naturally associated components. Accordingly, substantially pure proteins include eukaryotic proteins synthesized in *E. coli*, other prokaryotes, or any other organism in which they do not naturally occur.

The term "oligonucleotide", as used herein, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors, which, in turn, depend upon the ultimate function and use of the oligonucleotide. The term "primer", as used herein, refers to an oligonucleotide, whether occurring naturally (as in a purified restriction digest) or produced synthetically, and which is capable of initiating synthesis of a strand complementary to a nucleic acid when placed under appropriate conditions, i.e., in the presence of nucleotides and an inducing agent, such as a DNA polymerase, and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the

primer will depend upon many factors, including temperature, sequence and/or homology of primer and the method used. For example, in diagnostic applications, the oligonucleotide primer typically contains 15–25 or more nucleotides, depending upon the complexity of the target sequence, although it may contain fewer nucleotides.

The primers herein are selected to be “substantially” complementary to particular target DNA sequences. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment (i.e., containing a restriction site) may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence to hybridize therewith and form the template for synthesis of the extension product.

The probe to which the DNA of the invention hybridizes preferably consists of a sequence of at least 20 consecutive nucleotides, more preferably 40 nucleotides, even more preferably 50 nucleotides, and most preferably 100 nucleotides or more (up to 100%) of the coding sequence of the nucleotides listed in SEQ ID No. 29 or the complement thereof. Such a probe is useful for detecting expression of PUMP-1 in a cell by a method including the steps of (a) acting mRNA obtained from the cell with a labeled PUMP-1 hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

By “high stringency” is meant DNA hybridization and wash conditions characterized by high temperature and low salt concentration, e.g., wash conditions of 65° C. at a salt concentration of approximately 0.1×SSC, or the functional equivalent thereof. For example, high stringency conditions may include hybridization at about 42° C. in the presence of about 50% formamide; a first wash at about 65° C. with about 2×SSC containing 1% SDS; followed by a second wash at about 65° C. with about 0.1×SSC.

The DNA may have at least about 70% sequence identity to the coding sequence of the nucleotides listed in SEQ ID No. 29, preferably at least 75% (e.g., at least 80%); and most preferably at least 90%. The identity between two sequences is a direct function of the number of matching or identical positions. When a position in both of the two sequences is occupied by the same monomeric subunit, e.g., if a given position is occupied by an adenine in each of two DNA molecules, then they are identical at that position. For example, if 7 positions in a sequence 10 nucleotides in length are identical to the corresponding positions in a second 10-nucleotide sequence, then the two sequences have 70% sequence identity. The length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 100 nucleotides. Sequence identity is typically measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group (GCG), University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705).

The present invention comprises a vector comprising a DNA sequence which encodes a human PUMP-1 protein, wherein said vector is capable of replication in a host, and comprises, in operable linkage: a) an origin of replication; b) a promoter; and c) a DNA sequence coding for said PUMP-1 protein. Preferably, the vector of the present invention

contains a portion of the DNA sequence shown in SEQ ID No. 29. Vectors may be used to amplify and/or express nucleic acid encoding a PUMP-1 protein or fragment thereof.

In addition to substantially full-length proteins, the invention also includes fragments (e.g., antigenic fragments) of the PUMP-1 protein. As used herein, “fragment,” as applied to a polypeptide, will ordinarily be at least 10 residues, more typically at least 20 residues, and preferably at least 30 (e.g., 50) residues in length, but less than the entire, intact sequence. Fragments of the PUMP-1 protein can be generated by methods known to those skilled in the art, e.g., by enzymatic digestion of naturally occurring or recombinant PUMP-1 protein, by recombinant DNA techniques using an expression vector that encodes a defined fragment of PUMP-1, or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of PUMP-1 (e.g., binding to an antibody specific for PUMP-1) can be assessed by methods described herein. Purified PUMP-1 or antigenic fragments of PUMP-1 can be used to generate new antibodies or to test existing antibodies (e.g., as positive controls in a diagnostic assay) by employing standard protocols known to those skilled in the art. Included in this invention is polyclonal antisera generated by using PUMP-1 or a fragment of PUMP-1 as the immunogen in, e.g., rabbits. Standard protocols for monoclonal and polyclonal antibody production known to those skilled in this art are employed. The monoclonal antibodies generated by this procedure can be screened for the ability to identify recombinant PUMP-1 cDNA clones, and to distinguish them from other cDNA clones.

Further included in this invention are PUMP-1 proteins which are encoded, at least in part, by portions of SEQ ID No. 29, e.g., products of alternative mRNA splicing or alternative protein processing events, or in which a section of PUMP-1 sequence has been deleted. The fragment, or the intact PUMP-1 polypeptide, may be covalently linked to another polypeptide, e.g., one which acts as a label, a ligand or a means to increase antigenicity.

The invention also includes a polyclonal or monoclonal antibody which specifically binds to PUMP-1. The invention encompasses not only an intact monoclonal antibody, but also an immunologically-active antibody fragment, e.g., a Fab or (Fab)₂ fragment; an engineered single chain Fv molecule; or a chimeric molecule, e.g., an antibody which contains the binding specificity of one antibody, e.g., of murine origin, and the remaining portions of another antibody, e.g., of human origin.

In one embodiment, the antibody, or a fragment thereof, may be linked to a toxin or to a detectable label, e.g., a radioactive label, non-radioactive isotopic label, fluorescent label, chemiluminescent label, paramagnetic label, enzyme label, or colorimetric label. Examples of suitable toxins include diphtheria toxin, *Pseudomonas* exotoxin A, ricin, and cholera toxin. Examples of suitable enzyme labels include malate hydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholinesterase, etc. Examples of suitable radioisotopic labels include ³H, ¹²⁵I, ¹³¹I, ³²P, ³⁵S, ¹⁴C, etc.

Paramagnetic isotopes for purposes of in vivo diagnosis can also be used according to the methods of this invention. There are numerous examples of elements that are useful in

magnetic resonance imaging. For discussions on in vivo nuclear magnetic resonance imaging, see, for example, Schaefer et al., (1989) *JACC* 14, 472-480; Shreve et al., (1986) *Magn. Reson. Med.* 3, 336-340; Wolf, G. L., (1984) *Physiol. Chem. Phys. Med. NMR* 16, 93-95; Wesbey et al., (1984) *Physiol. Chem. Phys. Med. NMR* 16, 145-155; Runge et al., (1984) *Invest. Radiol.* 19, 408-415. Examples of suitable fluorescent labels include a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an ophthaldehyde label, a fluorescamine label, etc. Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, an aequorin label, etc.

Those of ordinary skill in the art will know of other suitable labels which may be employed in accordance with the present invention. The binding of these labels to antibodies or fragments thereof can be accomplished using standard techniques commonly known and used by those of ordinary skill in the art. Typical techniques are described by Kennedy et al., (1976) *Clin. Chim. Acta* 70, 1-31; and Schurs et al., (1977) *Clin. Chim. Acta* 81, 1-40. Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method. All of these methods are incorporated by reference herein.

Also within the invention is a method of detecting PUMP-1 protein in a biological sample, which includes the steps of contacting the sample with the labeled antibody, e.g., radioactively tagged antibody specific for PUMP-1, and determining whether the antibody binds to a component of the sample. Antibodies to the PUMP-1 protein can be used in an immunoassay to detect increased levels of PUMP-1 protein expression in tissues suspected of neoplastic transformation. These same uses can be achieved with Northern blot assays and analyses.

As described herein, the invention provides a number of diagnostic advantages and uses. For example, the PUMP-1 protein is useful in diagnosing cancer in different tissues since this protein is highly overexpressed in tumor cells. Antibodies (or antigen-binding fragments thereof) which

bind to an epitope specific for PUMP-1 are useful in a method of detecting PUMP-1 protein in a biological sample for diagnosis of cancerous or neoplastic transformation. This method includes the steps of obtaining a biological sample (e.g., cells, blood, plasma, tissue, etc.) from a patient suspected of having cancer, contacting the sample with a labeled antibody (e.g., radioactively tagged antibody) specific for PUMP-1, and detecting the PUMP-1 protein using standard immunoassay techniques such as an ELISA. Antibody binding to the biological sample indicates that the sample contains a component which specifically binds to an epitope within PUMP-1.

Likewise, a standard Northern blot assay can be used to ascertain the relative amounts of PUMP-1 mRNA in a cell or tissue obtained from a patient suspected of having cancer, in accordance with conventional Northern hybridization techniques known to those of ordinary skill in the art. This Northern assay uses a hybridization probe, e.g., radiolabelled PUMP-1 cDNA, either containing the full-length, single stranded DNA having a sequence complementary to SEQ ID No. 29, or a fragment of that DNA sequence at least 20 (preferably at least 30, more preferably at least 50, and most preferably at least 100 consecutive nucleotides in length). The DNA hybridization probe can be labeled by any of the many different methods known to those skilled in this art.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion:

EXAMPLE 1

Amplification of Serine Proteases Using Redundant and Specific Primers

Only cDNA preparations deemed free of genomic DNA were used for gene expression analysis. Redundant primers were prepared for serine proteases, metallo-proteases and cysteine protease. The primers were synthesized to consensus sequences of to amino acid surrounding the catalytic triad for serine proteases, viz. histidine . . . aspartate . . . and serine. The sequences of both sense (histidine & aspartate) and antisense (aspartate and serine) redundant primers are shown in Table 2.

TABLE 2

PC Primers	5'→3'	SEQ ID No.
<u>Redundant Primers:</u>		
Serine Protease (histidine) = S1	tgggtigtiaicgcigcica(ct)t	1
Serine Protease (aspartic acid) = AS1	a(ag)ia(ag)igciatitcitticc	2
Serine Protease (serine) = AS11	a(ag)iggicccici(cg)(ta)(ag)tcicc	3
Cysteine Protease - sense	ca(ag)ggica(ag)tg(ct)ggi(ta)(cg)itg(ct)tg	4
Cysteine Protease - antisense	taicccic(ag)tt(ag)caicc(ct)tc	5
Metallo Protease - sense	cci(ac)gitg(tc)ggi(ga)(ta)icciga	6
Metallo Protease - antisense	tt(ag)tgicciai(ct)tc(ag)tg	7
<u>Specific Primers:</u>		
Serine Protease (hepsin) = sense	tgtcccgatggcgagtgttt	8
Serine Protease (hepsin) = antisense	cctgttgccatagtactgc	9
Serine Protease (SCCE) = sense	agatgaatgagtacaccgtg	10

TABLE 2-continued

PC Primers	5'→3'	SEQ ID No.
Serine Protease (SCCE) = antisense	ccagtaagtcccttgtaaacc	11
Serine Protease (Comp B) = sense	aaggacacgagagctgtat	12
Serine Protease (Comp B) = antisense	aagtggtagttggaggaagc	13
Serine Protease (Protease M) = sense	ctgtgatccaccctgactat	20
Serine Protease (Protease M) = antisense	cagggtgatgtatgcacact	21
Serine Protease (TADG12) = sense (Ser10-s)	gcgcactgtgtttatgagat	22
Serine Protease (TADG12) = antisense (Ser10-as)	ctctttggcttgacttgct	23
Serine Protease (TADG13) = sense	tgaggacatcattatgcac	24
Serine Protease (TADG13) = antisense	caagttttcccataattgg	25
Serine Protease (TADG14) = sense	acagtacgcctgggagacca	26
Serine Protease (TADG14) = antisense	ctgagacggtgcaattctgg	27
Cysteine Protease (Cath-L) = sense	attggagagagaaggctac	14
Cysteine Protease (Cath-L) = antisense	cttgggattgtacttacagg	15
Metallo Protease (PUMP1) = sense	cttccaaagtggtcacctac	16
Metallo Protease (PUMP1) = antisense	ctagactgctaccatccgtc	17

EXAMPLE 2

Carcinoma Tissue

Several protease entities were identified and subcloned from PCR amplification of cDNA derived from serous cystadenocarcinomas. Therefore, the proteases described herein are reflective of surface activities for this type of carcinoma, the most common form of ovarian cancer. Applicant has also shown PCR amplification bands unique to the mucinous tumor type and the clear cell type of similar base pair size. About 20–25% of ovarian cancers are classified as either mucinous, clear cell, or endometrioid.

EXAMPLE 3

Ligation, Transformation and Sequencing

To determine the identity of the PCR products, all the appropriate bands were ligated into Promega T-vector plasmid and the ligation product was used to transform JM109 cells (Promega) grown on selective media. After selection and culturing of individual colonies, plasmid DNA was isolated by means of the WIZARD MINIPREP™ DNA purification system (Promega). Inserts were sequenced using a Prism Ready Reaction Dydeoxy Terminators cycle sequencing kit (Applied Biosystems). Residual dye terminators were removed from the completed sequencing reaction using a CENTRISEP SPIN™ column (Princeton Separation), and samples were loaded into an Applied Biosystems Model 373A DNA sequencing system. The results of subcloning and sequencing for the serine protease primers are summarized in Table 3.

TABLE 3

Serine protease candidates Subclone	Primer Set	Gene Candidate
1	His-Ser	hepsin
2	His-Ser	SCCE
3	His-Ser	Compliment B
4	His-Asp	Cofactor 1

TABLE 3-continued

Serine protease candidates Subclone	Primer Set	Gene Candidate
5	His-Asp	TADG 12*
6	His-Ser	TADG 13*
7	His-Ser	TADG 14*
8	His-Ser	Protease M
9	His-Ser	TADG 15*

*indicates novel proteases

EXAMPLE 4

Cloning and Characterization

Cloning and characterization of new gene candidates was undertaken to expand the panel representative of extracellular proteases specific for ovarian carcinoma subtypes. Sequencing of the PCR products derived from tumor cDNA confirms the potential candidacy of these genes. The three novel genes all have conserved residues within the catalytic triad sequence consistent with their membership in the serine protease family.

Applicant compared the PCR products amplified from normal and carcinoma cDNAs using sense-histidine and antisense-aspartate as well as sense-histidine and antisense-serine. The anticipated PCR products of approximately 200 bp and 500 bp for those pairs of primers were observed (aspartate is approximately 50–70 amino acids downstream from histidine, and serine is about 100–150 amino acids toward the carboxy end from histidine).

FIG. 1 shows a comparison of PCR products derived from normal and carcinoma cDNA as shown by staining in an agarose gel. Two distinct bands in lane 2 were present in the primer pair sense-His/antisense ASP (AS1) and multiple bands of about 500 bp are noted in the carcinoma lane for the sense-His/antisense-Ser (AS2) primer pairs in lane 4.

EXAMPLE 5

Quantitative PCR

The mRNA overexpression of PUMP-1 was detected and determined using quantitative PCR. Quantitative PCR was performed generally according to the method of Noonan et al. [*Proc.Natl.Acad.Sci.,USA*, 87:7160-7164 (1990)]. The following oligonucleotide primers were used:

PUMP-1:

forward 5'-CTTCCAAAGTGGTCACCTAC-3' (SEQ ID No. 16), and

reverse 5'-CTAGACTGCTACCATCCGTC-3' (SEQ ID No. 17);

and β -tubulin:

forward 5'-TGCAATTGACAACGAGGC-3' (SEQ ID No. 18), and

reverse 5'-CTGTCTTGA CATTGTTG-3' (SEQ ID No. 19).

β -tubulin was utilized as an internal control. The predicted sizes of the amplified genes were 250 bp for PUMP-1 and 454 bp for β -tubulin. The primer sequences used in this study were designed according to the cDNA sequences described by Leytus et al. [*Biochemistry*, 27, 1067-1074 (1988)] for PUMP-1, and Hall et al. [*Mol. Cell. Biol.*, 3, 854-862 (1983)] for β -tubulin. The PCR reaction mixture consisted of cDNA derived from 50 ng of mRNA converted by conventional techniques, 5 pmol of sense and antisense primers for both the PUMP-1 gene and the β -tubulin gene, 200 μ mol of dNTPs, 5 μ Ci of α -³²PdCTP and 0.25 units of Taq DNA polymerase with reaction buffer (Promega) in a final volume of 25 μ l. The target sequences were amplified in parallel with the β -tubulin gene. Thirty cycles of PCR were carried out in a Thermal Cycler (Perkin-Elmer Cetus). Each cycle of PCR included 30 sec of denaturation at 95° C., 30 sec of annealing at 63° C. and 30 sec of extension at 72° C. The PCR products were separated on 2% agarose gels and the radioactivity of each PCR product was determined by using a PhosphorImager™ (Molecular Dynamics). Student's t test was used for comparison of mean values.

Experiments comparing PCR amplification in normal ovary and ovarian carcinoma suggested overexpression and/or alteration in mRNA transcript in tumor tissues. Northern blot analysis of TADG-14 confirms a transcript size of 1.4 kb and data indicate overexpression in ovarian carcinoma (FIG. 2). Isolation and purification using both PCR and a specific 250 bp PCR product to screen positive plaques yielded a 1.2 kb clone of TADG-14. Other proteases were amplified by the same method using the appropriate primers from Table 2.

EXAMPLE 6

Tissue Bank

A tumor tissue bank of fresh frozen tissue of ovarian carcinomas as shown in Table 4 was used for evaluation. Approximately 100 normal ovaries removed for medical reasons other than malignancy were obtained from surgery and were available as controls.

TABLE 4

Ovarian cancer tissue bank				
Stage	Total	Stage I/II	Stage III/IV	No
<u>Serous</u>				
Malignant	166	15	140	8
LMP	16	9	7	0
Benign	12	0	0	12

TABLE 4-continued

Ovarian cancer tissue bank				
Stage	Total	Stage I/II	Stage III/IV	No
<u>Mucinous</u>				
Malignant	26	6	14	6
LMP	28	25	3	0
Benign	3	0	0	3
<u>Endometrioid</u>				
Malignant	38	17	21	0
LMP	2	2	0	0
Benign	0	0	0	0
<u>Other*</u>				
Malignant	61	23	29	9
LMP	0	0	0	0
Benign	5	0	0	5

*Other category includes the following tumor types: Brenner's tumor, thecoma, teratoma, fibrothecoma, fibroma, granulosa cell, clear cell, germ cell, mixed müllerian, stromal, undifferentiated, and dysgerminoma.

From the tumor bank, approximately 100 carcinomas were evaluated encompassing most histological sub-types of ovarian carcinoma, including borderline or low-malignant potential tumors and overt carcinomas. The approach included using mRNA prepared from fresh frozen tissue (both normal and malignant) to compare expression of genes in normal, low malignant potential tumors and overt carcinomas. The cDNA prepared from polyA⁺ mRNA was deemed to be genomic DNA-free by checking all preparations with primers that encompassed a known intron-exon splice site using both β -tubulin and p53 primers.

EXAMPLE 7

Northern Blots

Significant information can be obtained by examining the expression of these candidate genes by Northern blot. Analysis of normal adult multi-tissue blots offers the opportunity to identify normal tissues which may express the protease. Ultimately, if strategies for inhibition of proteases for therapeutic intervention are to be developed, it is essential to appreciate the expression of these genes in normal tissue if and when it occurs.

Significant information is expected from Northern blot analysis of fetal tissue. Genes overexpressed in carcinomas are often highly expressed in organogenesis. As indicated, the hepsin gene cloned from hepatoma cells and overexpressed in ovarian carcinoma is overtly expressed in fetal liver. Hepsin gene expression was also detected in fetal kidney, and therefore, could be a candidate for expression in renal carcinomas.

Northern panels for examining expression of genes in a multi-tissue normal adult as well as fetal tissue are commercially available (CLONTECH). Such evaluation tools are not only important to confirm the overexpression of individual transcripts in tumor versus normal tissues, but also provides the opportunity to confirm transcript size, and to determine if alternate splicing or other transcript alteration may occur in ovarian carcinoma.

EXAMPLE 8

Northern Blot Analysis

Northern blot analysis was performed as follows: 10 μ g of mRNA was loaded onto a 1% formaldehyde-agarose gel, electrophoresed and blotted onto a HyBond-N⁺™ nylon membrane (Amersham). ³²P-labeled cDNA probes were made using Prime-a-Gene Labeling System™ (Promega).

The PCR products amplified by specific primers were used as probes. Blots were prehybridized for 30 min and then hybridized for 60 min at 68° C. with ³²P-labeled cDNA probe in ExpressHyb™ Hybridization Solution (CLONTECH). Control hybridization to determine relative gel loading was accomplished using the β -tubulin probe.

EXAMPLE 9

PCR Products Corresponding to Serine, Cysteine and Metallo-proteases

Based on their unique expression in either low malignant potential tumors or carcinomas, PCR-amplified cDNA products were cloned and sequenced and the appropriate gene identified based upon nucleotide and amino acid sequences stored in the GCG and EST databases. FIGS. 3, 4 & 5 show the PCR product displays comparing normal and carcinomatous tissues using redundant primers for serine proteases (FIG. 3), for cysteine proteases (FIG. 4) and for metallo-proteases (FIG. 5). Note the differential expression in the carcinoma tissues versus the normal tissues. The proteases were identified using redundant cDNA primers (see Table 2) directed towards conserved sequences that are associated with intrinsic enzyme activity (for serine proteases, cysteine proteases and metallo-proteases) by comparing mRNA expression in normal, low malignant potential and overt ovarian carcinoma tissues according to Sakanari et al. [*Biochemistry* 86, 4863-4867 (1989)].

EXAMPLE 10

Serine Proteases

For the serine protease group, using the histidine domain primer sense, S1, in combination with antisense primer AS2, the following proteases were identified:

- (a) Hepsin, a trypsin-like serine protease cloned from hepatoma cells shown to be a cell surface protease essential for the growth of hepatoma cells in culture and highly expressed in hepatoma tumor cells (FIG. 3, lane 4);
- (b) Complement factor B protease (human factor IX), a protease involved in the coagulation cascade and associated with the production and accumulation of fibrin split products associated with tumor cells (FIG. 3, lane 4). Complement factor B belongs in the family of coagulation factors X (Christmas factor). As part of the intrinsic pathway, complement factor B catalyzes the proteolytic activation of coagulation factor X in the presence of Ca²⁺ phospholipid and factor VIIIa e5; and
- (c) A stratum corneum chymotryptic enzyme (SCCE) serine protease involved in desquamation of skin cells from the human stratum corneum (FIG. 3, lane 4). SCCE is expressed in keratinocytes of the epidermis and functions to degrade the cohesive structures in the cornified layer to allow continuous skin surface shedding.

EXAMPLE 11

Cysteine Proteases

In the cysteine protease group, using redundant sense and anti-sense primers for cysteine proteases, one unique PCR product was identified by overexpression in ovarian carcinoma when compared to normal ovarian tissue (FIG. 4, lanes 3-5). Cloning and sequencing this PCR product identified a sequence of Cathepsin L, which is a lysosomal cysteine protease whose expression and secretion is induced by malignant transformation, growth factors and tumor promoters. Many human tumors (including ovarian) express high

levels of Cathepsin L. Cathepsin L cysteine protease belongs in the stromolysin family and has potent elastase and collagenase activities. Published data indicates increased levels in the serum of patients with mucinous cystadenocarcinoma of the ovary. It has not heretofore been shown to be expressed in other ovarian tumors.

EXAMPLE 12

Metallo-proteases

- 10 Using redundant sense and anti-sense primers for the metallo-protease group, one unique PCR product was detected in the tumor tissue which was absent in normal ovarian tissue (FIG. 5, lanes 2-5). Subcloning and sequencing this product indicates it has complete homology in the appropriate region with the so-called PUMP-1 (MMP-7) gene. This zinc-binding metallo-protease is expressed as a proenzyme with a signal sequence and is active in gelatin and collagenase digestion. PUMP-1 has also been shown to be induced and overexpressed in 9 of 10 colorectal carcinomas compared to normal colon tissue, suggesting a role for this substrate in the progression of this disease.

EXAMPLE 13

Expression of PUMP-1

- 25 The expression of the metallo-protease PUMP-1 gene in 10 normal ovaries, 12 low malignant potential (LMP) tumors, and 32 ovarian carcinoma (both mucinous and serous type) by quantitative PCR using PUMP-1 -specific primers (see Table 2) was determined (primers directed toward the β -tubulin message were used as an internal standard) (Table 5). Using a cut-off level for overexpression of the mean for normal ovary +2SD, 9 of 12 LMP (75%) tumor cases and 26 of 32 (81%) carcinoma cases were above the cut-off value. PUMP-1 mRNA expression was significantly elevated in tumors compared to that in normal ovary for both LMP tumor ($p<0.05$) and carcinoma ($p<0.0001$). All 10 cases of normal ovaries showed relatively low levels of PUMP-1 mRNA expression.

- 40 Table 5 summarizes the data obtained on the histological type, stage, grade and mRNA overexpression of PUMP-1 in all the cases studied. Lymph node metastases were histopathologically proven in 5 cases and all these cases showed overexpression of PUMP-1. Also of note, all 5 cases of stage I carcinoma showed overexpression of PUMP-1. Overall, the expression ratio (mean \pm SD) for normal ovary was determined to be 0.084 \pm 0.065; for LMP tumors, 0.905 \pm 1.251; and for carcinomas, 0.663 \pm 0.630 (Table 6). From a histological point of view (Table 6), overexpression of PUMP-1 was observed in 21 of 26 (80.8%) serous tumors (6 of 7 LMP tumors and 15 of 19 carcinomas) and 8 of 12 (66.7%) mucinous tumors (3 of 5 LMP tumors and 5 of 7 carcinomas). For endometrioid and clear cell carcinomas, PUMP-1 was found to be overexpressed in all 6 cases examined.

TABLE 5

Patient Characteristics and Expression of PUMP-1 Gene				
Case	Histological type ^a	Stage/Grade	LN ^b	mRNA expression of PUMP-1 ^c
1	normal ovary			n
2	normal ovary			n
3	normal ovary			n
4	normal ovary			n
5	normal ovary			n

TABLE 5-continued

Patient Characteristics and Expression of PUMP-1 Gene				
Case	Histological type ^a	Stage/Grade	LN ^b	mRNA expression of PUMP-1 ^c
6	normal ovary			n
7	normal ovary			n
8	normal ovary			n
9	normal ovary			n
10	normal ovary			n
11	S adenoma (LMP)	1/1	N	4+
12	S adenoma (LMP)	1/1	NE	n
13	S adenoma (LMP)	1/1	NE	4+
14	S adenoma (LMP)	1/1	N	4+
15	S adenoma (LMP)	3/1	P	4+
16	S adenoma (LMP)	3/1	P	4+
17	S adenoma (LMP)	3/1	P	4+
18	M adenoma (LMP)	1/1	NE	n
19	M adenoma (LMP)	1/1	N	n
20	M adenoma (LMP)	1/1	N	4+
21	M adenoma (LMP)	1/1	NE	4+
22	M adenoma (LMP)	1/1	NE	4+
23	S carcinoma	1/2	N	4+
24	S carcinoma	1/3	N	4+
25	S carcinoma	3/1	NE	4+
26	S carcinoma	3/2	NE	4+
27	S carcinoma	3/2	P	4+
28	S carcinoma	3/2	NE	2+
29	S carcinoma	3/3	NE	n
30	S carcinoma	3/3	NE	4+
31	S carcinoma	3/3	NE	4+
32	S carcinoma	3/3	NE	4+
33	S carcinoma	3/3	N	2+
34	S carcinoma	3/3	NE	n
35	S carcinoma	3/3	NE	4+
36	S carcinoma	3/3	NE	4+
37	S carcinoma	3/3	NE	2+
38	S carcinoma	3/3	N	n
39	S carcinoma	3/2	NE	4+
40	S carcinoma	3/3	NE	2+
41	S carcinoma	3/2	NE	n
42	M carcinoma	1/2	N	4+
43	M carcinoma	2/2	NE	4+
44	M carcinoma	2/2	N	4+
45	M carcinoma	3/1	NE	4+
46	M carcinoma	3/2	NE	n
47	M carcinoma	3/2	NE	n
48	M carcinoma	3/3	NE	4+
49	E carcinoma	2/3	N	4+
50	E carcinoma	3/2	NE	4+
51	E carcinoma	3/3	NE	4+
52	C carcinoma	1/3	N	4+
53	C carcinoma	1/1	N	4+
54	C carcinoma	3/2	P	4+

^aS, serous; M, mucinous; E, endometrioid; C, clear cell; ^bLN, lymph node metastasis; P, positive; N, negative; NE, not examined; ^cn, normal range = mean \pm 2SD; 2+, mean +2SD to +4SD; 4+, mean +4SD or greater.

TABLE 6

Overexpression of PUMP-1 in normal ovaries and ovarian tumors			
Type	N	PUMP-1 Overexpression	Ratio of PUMP-1 to β -tubulin
Normal	10	0 (0%)	0.084 \pm 0.065
LMP	12	9 (75.0%)	0.905 \pm 1.251
Serous	7	6 (85.7%)	1.301 \pm 1.542
Mucinous	5	3 (60.0%)	0.351 \pm 0.269
Carcinomatous	32	26 (81.3%)	0.663 \pm 0.630
Serous	19	15 (78.9%)	0.675 \pm 0.774
Mucinous	7	5 (71.4%)	0.474 \pm 0.337
Endometrioid	3	3 (100%)	0.635 \pm 0.224
Clear Cell	3	3 (100%)	1.062 \pm 0.060

EXAMPLE 14

Expression of SCCE and PUMP-1

Studies using both SCCE-specific primers (FIG. 8) and PUMP-specific primers (FIG. 9) indicate overexpression of these proteases in ovarian carcinomas.

Examination of PUMP-1 antigen expression in normal and carcinomatous tissues by immunolocalization using both a peptide derived polyclonal antibody and a commercial monoclonal antibody (Calbiochem) confirmed the quantitative PCR data shown in Table 7. Little or no staining was observed in normal ovary (FIG. 20A), while intense tumor cell staining of secretory bodies could be detected in several mucinous tumors (e.g., FIG. 20B). Intense cytoplasmic staining was also observed in serous tumors (FIG. 20C), clear cell tumors (FIG. 20D) and a secreted product was most noticeable in endometrioid tumors (FIG. 20E).

TABLE 7

Expression of PUMP-1 protein by immunolocalization		
Histology	mRNA ^a	Protein ^b
Normal ovary	n	—
S Carcinoma	4+	+
S Carcinoma	4+	+
S Carcinoma	4+	+
S Carcinoma	4+	+
S Carcinoma	2+	+
S Carcinoma	n	—
S Carcinoma	4+	+
S Carcinoma	2+	+
S Carcinoma	n	—
M Carcinoma	n	—
C Carcinoma	4+	+
C Carcinoma	4+	+
C Carcinoma	4+	+

^amRNA expression of PUMP-1 (see Table 5). n = low or no transcript detected by quantitative PCR. 2+/4+ = overexpression of PUMP-1 transcription by more than 2SD or 4SD over the normal level of PUMP-1 in normal ovary. ^b+, >10% positive tumor cells; —, negative.

EXAMPLE 15

Summary of Known Proteases Detected Herein

Most of the proteases described herein were identified from the sense-His/antisense-Ser primer pair, yielding a 500 bp PCR product (FIG. 1, lane 4). Some of the enzymes are familiar, a short summary of each follows.

Hepsin

Hepsin is a trypsin-like serine protease cloned from hepatoma cells. Hepsin is an extracellular protease (the enzyme includes a secretion signal sequence) which is anchored in the plasma membrane by its amino terminal domain, thereby exposing its catalytic domain to the extracellular matrix. Hepsin has also been shown to be expressed in breast cancer cell lines and peripheral nerve cells. It has never before been associated with ovarian carcinoma. Specific primers for the hepsin gene were synthesized and the expression of hepsin examined using Northern blots of fetal tissue and ovarian tissue (both normal and ovarian carcinoma).

FIG. 10A shows that hepsin was expressed in fetal liver and fetal kidney as anticipated, but at very low levels or not at all in fetal brain and lung. FIG. 10B shows that hepsin was expressed in ovarian carcinomas of different histologic types, but not in normal ovary. The mRNA identified in both Northern blots was the appropriate size for the hepsin transcript. The expression of hepsin was examined in 10 normal ovaries and 44 ovarian tumors using specific primers to β -tubulin and hepsin in a quantitative PCR assay, and found it to be linear over 35 cycles. Expression is presented

as the ratio of ^{32}P -hepsin band to the internal control, the ^{32}P - β -tubulin band.

FIGS. 11A & 11B show hepsin expression in normal (N), mucinous (M) and serous (S) low malignant potential (LMP) tumors and carcinomas (CA). FIG. 11A shows quantitative PCR of hepsin and internal control β -tubulin. FIG. 11B shows a bar graph of expression of PUMP-1 in 10 normal ovaries and 44 ovarian carcinoma samples.

Hepsin mRNA is highly overexpressed in most histopathologic types of ovarian carcinomas including some low malignant potential tumors (see FIGS. 11A & 11B). Most noticeably, hepsin is highly expressed in serous, endometrioid and clear cell tumors tested. It is highly expressed in some mucinous tumors, but it is not overexpressed in the majority of such tumors. Stratum corneum chymotrypsin enzyme (SCCE)

The PCR product identified was the catalytic domain of the sense-His/antisense-Ser of the SCCE enzyme. This extracellular protease was cloned, sequenced and shown to be expressed on the surface of keratinocytes in the epidermis. SCCE is a chymotrypsin-like serine protease whose function is suggested to be in the catalytic degradation of intercellular cohesive structures in the stratum corneum layer of the skin. This degradation allows continuous shedding (desquamation) of cells from the skin surface. The subcellular localization of SCCE is in the upper granular layer in the stratum corneum of normal non-palmoplantar skin and in the cohesive parts of hypertrophic plantar stratum corneum. SCCE is exclusively associated with the stratum corneum and has not so far been shown to be expressed in any carcinomatous tissues.

Northern blots were probed with the PCR product to determine expression of SCCE in fetal tissue and ovarian carcinoma (FIGS. 12A & 12B). Noticeably, detection of SCCE messenger RNA on the fetal Northern was almost non-existent (a problem with the probe or the blot was excluded by performing the proper controls). A faint band appeared in fetal kidney. On the other hand, SCCE mRNA is abundant in the ovarian carcinoma mRNA (FIG. 12B). Two transcripts of the correct size are observed for SCCE. The same panel of cDNA used for hepsin analysis was used for SCCE expression.

No SCCE expression was detected in the normal ovary lane of the Northern blot. A comparison of all candidate genes, including a loading marker (β -tubulin), was shown to confirm that this observation was not a result of a loading bias. Quantitative PCR using SCCE primers, along with β -tubulin internal control primers, confirmed the overexpression of SCCE mRNA in carcinoma of the ovary with no expression in normal ovarian tissue (FIG. 13).

FIG. 13A shows a comparison using quantitative PCR of SCCE cDNA from normal ovary and ovarian carcinomas. FIG. 13B shows the ratio of SCCE to the β -tubulin internal standard in 10 normal and 44 ovarian carcinoma tissues. Again, it is observed that SCCE is highly overexpressed in ovarian carcinoma cells. It is also noted that some mucinous tumors overexpress SCCE, but the majority do not.

Protease M

Protease M was identified from subclones of the His—ser primer pair. This protease was first cloned by Anisowicz, et al., [*Molecular Medicine*, 2, 624–636 (1996)] and shown to be overexpressed in breast and ovarian carcinomas. A preliminary evaluation indicates that this enzyme is overexpressed in ovarian carcinoma (FIG. 14).

Cofactor I and Complement Factor B

Several serine proteases associated with the coagulation pathway were also subcloned. Examination of normal and

ovarian carcinomas by quantitative PCR for expression of these enzymes, it was noticeable that this mRNA was not clearly overexpressed in ovarian carcinomas when compared to normal ovarian tissue. It should be noted that the same panel of tumors was used for the evaluation of each candidate protease.

EXAMPLE 16

Summary of Previously Unknown Proteases Detected Herein

TADG-12

TADG-12 was identified from the primer pairs, sense-His/antisense-Asp (see FIG. 1, lanes 1 & 2). Upon subcloning both PCR products in lane 2, the 200 bp product had a unique protease-like sequence not included in GenBank. This 200 bp product contains many of the conserved amino acids common for the His-Asp domain of the family of serine proteins. The second and larger PCR product (300 bp) was shown to have a high degree of homology with TADG-12 (His-Asp sequence), but also contained approximately 100 bp of unique sequence. Synthesis of specific primers and the sequencing of the subsequent PCR products from three different tumors demonstrated that the larger PCR product (present in about 50% of ovarian carcinomas) includes an insert of about 100 bp near the 5' end (and near the histidine) of the sequence. This insert may be a retained genomic intron because of the appropriate position of splice sites and the fact that the insert does not contain an open reading frame (see FIG. 15). This suggests the possibility of a splice site mutation which gives rise to retention of the intron, or a translocation of a sequence into the TADG-12 gene in as many as half of all ovarian carcinomas.

TADG-13 and TADG-14

Specific primers were synthesized for TADG-13 and TADG-14 to evaluate expression of genes in normal and ovarian carcinoma tissue. Northern blot analysis of ovarian tissues indicates the transcript for the TADG-14 gene is approximately 1.4 kb and is expressed in ovarian carcinoma tissues (FIG. 16A) with no noticeable transcript presence in normal tissue. In quantitative PCR studies using specific primers, increased expression of TADG-14 in ovarian carcinoma tissues was noted compared to a normal ovary (FIG. 16B). The presence of a specific PCR product for TADG-14 in both an HeLa library and an ovarian carcinoma library was also confirmed. Several candidate sequences corresponding to TADG-14 have been screened and isolated from the HeLa library.

Clearly from sequence homology, these genes fit into the family of serine proteases. TADG-13 and -14 are, however, heretofore undocumented genes which the specific primers of the invention allow to be evaluated in normal and tumor cells, and with which the presence or absence of expression of these genes is useful in the diagnosis or treatment selection for specific tumor types.

PUMP-1

In a similar strategy using redundant primers to metal binding domains and conserved histidine domains, a differentially expressed PCR product identical to matrix metalloprotease 7 (MMP-7) was identified, herein called PUMP-1. Using specific primers for PUMP-1, PCR produced a 250 bp product for Northern blot analysis.

To confirm the results of quantitative PCR and to identify the appropriate transcript size for PUMP-1, Northern blot hybridization was performed using representative samples of each histological type of carcinoma. As shown in FIG. 17A, Northern blot hybridization with a ^{32}P -labeled PUMP-1 probe revealed an intense band in carcinoma cases and no visible band in normal ovary. The size of the

PUMP-1 transcript in carcinoma cases was approximately 1.1 Kb. Among normal human fetal tissues examined, fetal lung and fetal kidney showed increased transcript expression (FIG. 17B), on the other hand, PUMP-1 expression was not observed or was expressed at very low levels in normal human adult tissues, including spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocyte (FIG. 17C).

Quantitative PCR comparing normal versus ovarian carcinoma expression of the PUMP-1 mRNA indicates that this gene is highly expressed in serous carcinomas, including most low malignant serous tumors, and is, again, expressed to a lesser extent in mucinous tumors (see FIGS. 18A & 18B). PUMP-1, however, is so far the protease most frequently found overexpressed in mucinous tumors (See Table 8).

Cathepsin-L

Using redundant cysteine protease primers to conserved domains surrounding individual cysteine and histidine residues, the cathepsin-L protease was identified in several serous carcinomas. An initial examination of the expression of cathepsin L in normal and ovarian tumor tissue indicates that transcripts for the cathepsin-L protease are present in both normal and tumor tissues (FIG. 21). However, its presence or absence in combination with other proteases of the present invention permits identification of specific tumor types and treatment choices.

EXAMPLE 17

Summary of Data

Redundant primers to conserved domains of serine, metallo-, and cysteine proteases have yielded a set of genes whose mRNAs are overexpressed in ovarian carcinoma. The genes which are clearly overexpressed include the serine proteases hepsin, SCCE, protease MTADG12, TADG14 and the metallo-protease PUMP-1 (see FIG. 21 and Table 8). Northern blot analysis of normal and ovarian carcinoma tissues, summarized in FIG. 14, indicated overexpression of hepsin, SCCE, PUMP-1 and TADG-14. A β -tubulin probe to control for loading levels was included.

TABLE 8

Overexpression of proteases in ovarian tumors					
Type	N	PUMP-1	SCCE	Pump-1	Prot M
Normal	10	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/10)
LMP	12	58.3% (7/12)	66.7% (8/12)	75.0% (9/12)	75% (9/12)
Serous	7	85.7% (6/7)	85.7% (6/7)	85.7% (6/7)	100% (7/7)
mucinous	5	20.0% (1/5)	40.0% (2/5)	60% (3/5)	40.0% (2/5)
Carcinoma	32	84.4% (27/32)	78.1% (25/32)	81.3% (26/32)	90.6% (29/32)
serous	19	94.7% (18/19)	89.5% (17/19)	78.9% (15/19)	94.7% (18/19)
mucinous	7	42.9% (3/7)	28.6% (2/7)	71.4% (5/7)	85.7% (6/7)
endometr.	3	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
clear cell	3	100% (3/3)	100% (3/3)	100% (3/3)	67.7% (2/3)

EXAMPLE 18

Implications

For the most part, these proteins previously have not been associated with the extracellular matrix of ovarian carcinoma cells. No panel of proteases which might contribute to the growth, shedding, invasion and colony development of metastatic carcinoma has been previously described, including the three new candidate serine proteases which are herein disclosed. The establishment of an extracellular protease panel associated with either malignant growth or

malignant potential offers the opportunity for the identification of diagnostic or prognostic markers and for therapeutic intervention through inhibition or down regulation of these proteases.

The availability of the instant gene-specific primers coding for the appropriate region of tumor specific proteases allows for the amplification of a specific cDNA probe using Northern and Southern analysis, and their use as markers to detect the presence of the cancer in tissue. The probes also allow more extensive evaluation of the expression of the gene in normal ovary versus low malignant potential tumor, as well as both high- and low-stage carcinomas. The evaluation of a panel of fresh frozen tissue from all the carcinoma subtypes (Table 4) allowed the determination of whether a protease is expressed predominantly in early stage disease or within specific carcinoma subtypes. It was also determined whether each genes' expression is confined to a particular stage in tumor progression and/or is associated with metastatic lesions. Detection of specific combinations of proteases is an identifying characteristic of the specific tumor types and yields valuable information for diagnoses and treatment selection. Particular tumor types may be more accurately diagnosed by the characteristic expression pattern of each specific tumor.

EXAMPLE 19

Antisense PUMP-1

PUMP-1 is cloned and expressed in the opposite orientation such that an antisense RNA molecule (SEQ ID No. 28) is produced. For example, the antisense RNA is used to hybridize to the complementary RNA in the cell and thereby inhibit translation of PUMP-1 RNA into protein.

EXAMPLE 20

Peptide Ranking

For vaccine or immune stimulation, individual 9-mers to 11-mers of the PUMP-1 protein were examined to rank the binding of individual peptides to the top 8 haplotypes in the general population (Parker et al., (1994)). The computer program used for this analyses can be found at <http://www-bimas.dcrf.nih.gov/molbio/hla_bind/>. Table 9 shows the peptide ranking based upon the predicted half-life of each peptide's binding to a particular HLA allele. A larger half-life indicates a stronger association with that peptide and the particular HLA molecule. The PUMP-1 peptides that strongly bind to an HLA allele are putative immunogens, and are used to inoculate an individual against PUMP-1.

TABLE 9

<u>PUMP-1 peptide ranking</u>				
HLA Type & Ranking	Start	Peptide	Predicted Dissociation _{1/2}	SEQ ID No.
<u>HLA A0201</u>				
1	208	FLYAATHL	314.455	30
2	134	NMWGKEIPL	128.056	31
3	81	IMQKPRCGV	85.394	32
4	10	CLLPGSLAL	79.041	33
5	60	KEMQKFFGL	59.278	34
6	203	SLGINFLYA	51.916	35
7	73	MLNSRVIEI	40.792	36
8	4	TVLCAVCLL	15.907	37
9	132	ALNMWGKEI	10.433	38
10	109	VTYRIVSYT	7.122	39
11	127	RLVSKALNM	4.968	40
12	154	IMIGFARGA	4.636	41
13	43	LYDSETKN	4.497	42
14	140	IPLHFRKVV	4.449	43
15	146	KVVWGTADI	3.195	44
16	36	QDYLRKFYL	3.029	45
17	2	RLTVLCAVC	2.037	46
18	201	GSSLGINFL	1.764	47
19	70	ITGMLNSRV	1.642	48
20	205	GINFLYAAT	1.537	49
<u>HLA A0205</u>				
1	4	TVLCAVCLL	84.000	50
2	208	FLYAATHL	63.000	51
3	60	KEMQKFFGL	21.168	52
4	10	CLLPGSLAL	21.000	53
5	134	NMWGKEIPL	21.000	54
6	8	AVCLLPGSL	14.000	55
7	146	KVVWGTADI	6.000	56
8	73	MLNSRVIEI	3.400	57
9	81	IMQKPRCGV	3.400	58
10	66	FGLPITGML	3.150	59
11	147	VVWGTADIM	2.550	60
12	212	ATHELGHSL	2.100	61
13	178	HAFAPGTGL	2.100	62
14	205	GINFLYAAT	2.000	63
15	22	QEAGGMSEL	1.960	64
16	112	RIVSYTRDL	1.680	65
17	167	YPFDGPGNT	1.350	66
18	3	LTVLCAVCL	1.190	67
19	140	IPLHFRKVV	1.020	68
20	109	VTYRIVSYT	1.020	69
<u>HLA A1</u>				
1	225	SSDPNAVMY	750.000	70
2	78	VIEIMQKPR	9.000	71
3	198	WTDGSSLGI	6.250	72
4	238	NGDPQNFKL	6.250	73
5	92	VAEYSLFPN	4.500	74
6	35	AQDYLRKFY	3.750	75
7	202	SSLGINFLY	3.750	76
8	46	DSETKNANS	2.700	77
9	87	CGVPDVAEY	2.500	78
10	248	QDDIKGIQK	2.500	79
11	27	MSELQWEQA	1.350	80
12	150	GTADIMIGF	1.250	81
13	123	ITVDRLVSK	1.000	82
14	108	VVTYRIVSY	1.000	83
15	54	SLEAKLKEM	0.900	84
16	163	HGDSYPPFDG	0.625	85
17	10	CLLPGSLAL	0.500	86
18	151	TADIMIGFA	0.500	87
19	138	KEIPLHFRK	0.500	88
20	137	GKEIPLHFR	0.450	89
<u>HLA A24</u>				
1	115	SYTRDLPHI	50.000	90
2	112	RIVSYTRDL	12.000	91
3	66	FGLPITGML	10.080	92
4	37	DYLKRFYLY	9.000	93
5	51	NANSLEAKL	7.920	94
6	166	SYPFKGPGN	7.500	95

TABLE 9-continued

<u>PUMP-1 peptide ranking</u>				
HLA Type & Ranking	Start	Peptide	Predicted Dissociation _{1/2}	SEQ ID No.
<u>HLA B7</u>				
7	10	CLLPGSLAL	7.200	96
8	4	TVLCAVCLL	6.000	97
9	31	QWEQAQDYL	6.000	98
10	3	LTVLCAVCL	6.000	99
11	212	ATHELGHSL	5.760	100
12	238	NGDPQNFKL	5.280	101
13	44	LYDSETKNA	5.000	102
14	235	TYGNGDPQN	5.000	103
15	243	NFKLSQDDI	5.000	104
16	8	AVCLLPGSL	4.800	105
17	12	LPGSLALPL	4.800	106
18	58	KLKEMQKFF	4.800	107
19	201	GSSLGINFL	4.800	108
20	208	FLYAATHL	4.400	109
<u>HLA B8</u>				
1	120	LPHITVDRL	80.000	110
2	12	LPGSLALPL	80.000	111
3	8	AVCLLPGSL	60.000	112
4	84	KPRCGVPDV	40.000	113
5	89	VPDVAEYSL	24.000	114
6	4	TVLCAVCLL	20.000	115
7	178	HAFAPGTGL	18.000	116
8	51	NANSLEAKL	12.000	117
9	212	ATHELGHSL	12.000	118
10	140	IPLHFRKVV	6.000	119
11	147	VVWGTADIM	5.000	120
12	208	FLYAATHL	4.000	121
13	101	SPKWTISKVV	4.000	122
14	10	CLLPGSLAL	4.000	123
15	3	LTVLCAVCL	4.000	124
16	201	GSSLGINFL	4.000	125
17	134	NMWGKEIPL	4.000	126
18	112	RIVSYTRDL	4.000	127
19	125	VDRLVSKAL	4.000	128
20	66	FGLPITGML	4.000	129
<u>HLA B2702</u>				
1	134	NMWGKEIPL	4.000	130
2	56	EAKLKEMQK	3.200	131
3	101	SPKWTISKVV	2.400	132
4	73	MLNSRVIEI	2.000	133
5	84	KPRCGVPDV	1.200	134
6	127	RLVSKALNM	1.000	135
7	105	TSKVVTYRI	1.000	136
8	51	NANSLEAKL	0.800	137
9	12	LPGSLALPL	0.800	138
10	120	LPHITVDRL	0.800	139
11	178	HAFAPGTGL	0.800	140
12	54	SLEAKLKEM	0.400	141
13	10	CLLPGSLAL	0.400	142
14	208	FLYAATHL	0.400	143
15	125	VDRLVSKAL	0.400	144
16	158	FARGAHGDS	0.400	145
17	36	QDYLRKFYL	0.400	146
18	212	ATHELGHSL	0.300	147
19	116	YTRDLPHIT	0.300	148
20	62	MQKFFGLPI	0.300	149
<u>HLA B2702</u>				
1	159	ATGAHGDSY	200.000	150
2	30	LQWEQAQDY	100.00	151
3	196	ERWTDGSSL	90.000	152
4	40	KRFYLYDSE	30.000	153
5	1	MRLTVLCAV	20.000	154
6	144	FRKVVWGTA	20.000	155
7	117	TRDLPHITV	20.000	156
8	134	NMWGKEIPL	7.500	157
9	96	SLFPNSPKW	7.500	158
10	62	MQKFFGLPI	6.000	159
11	35	AQDYLRKFY	6.000	160
12	208	FLYAATHL	4.500	161
13	76	SRVIEIMQK	4.000	162
14	126	DRLVSKALN	3.000	163

TABLE 9-continued

PUMP-1 peptide ranking				
HLA Type & Ranking	Start	Peptide	Predicted Dissociation _{1/2}	SEQ ID No.
15	60	KEMQKFFGL	2.700	164
16	58	KLKEMQKFF	2.700	165
17	256	KLYGKRSNS	2.250	166
18	85	PRCGVPDVA	2.000	167
19	111	YRIVSYTRD	2.000	168
20	178	HAFAPGTGL	1.500	169
HLA B4403				
1	87	CGVPDVAEY	36.000	170
2	202	SSLGINFLY	27.000	171
3	79	IEIMQKPRC	20.000	172
4	60	KEMQKFFGL	18.000	173
5	225	SSDPNAVMY	18.000	174
6	47	SETKNANSL	12.000	175
7	195	DERWTDGSS	12.000	176
8	214	HELGHSLGM	12.000	177
9	22	QEAGGMSEL	12.000	178
10	249	DDIKGIQKL	11.250	179
11	93	AEYSLFPNS	8.000	180
12	138	KEIPLHFRK	6.000	181
13	184	TGLGGDAHF	3.000	182
14	200	DGSSLGINF	3.000	183
15	35	AQDYLKRFY	3.000	184
16	34	QAQDYLKRF	2.250	185
17	30	LQWEQAQDY	2.250	186
18	250	DIKGIQKLY	2.025	187

TABLE 9-continued

PUMP-1 peptide ranking				
HLA Type & Ranking	Start	Peptide	Predicted Dissociation _{1/2}	SEQ ID No.
19	150	GTADIMIGF	2.000	188
20	37	DYLKRFYLY	1.800	189

10

Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. Further, these patents and publications are incorporated by reference herein to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

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 uuuaaagccu uugacacuaa ucgauccacu guaaauugcg guaaugucug 700
 aguauaugau acgauccugu aggugaccac uuuggaaguc cauuuugggc 750
 uauuuggaaa uagugaguau ucugcaacau cuggcacucc acaucugggc 800
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 gccaaagaau uuugcaucu ccuugaguuu ggcuucuaaa cuguuggcau 900
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 uguucccacu guagcucacu caugccuccc gccuccugag gcagcggcag 1000
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 uagcugccgu ccagagacaa uuguucuu 1078

<210> SEQ ID NO 29
 <211> LENGTH: 1078
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: cDNA of PUMP-1

<400> SEQUENCE: 29

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gaggcatgag tgagctacag tgggaacagg ctcaggacta tctcaagaga	150
ttttatctct atgactcaga aacaaaaaat gccaacagtt tagaagccaa	200
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actcccgct catagaaata atgcagaagc ccagatgttg agtgccagat	300
gttgacagaat actcactatt tccaaatagc ccaaaatgga cttccaaagt	350
ggtcacctac aggatcgtat catatactcg agacttaccg catattacag	400
tggatcgatt agtgtcaaag gctttaaaca tgtggggcaa agagatcccc	450
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cctgtatgct gcaactcatg aacttgacca ttctttgggt atgggacatt	700
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tggaaagaga agtaattcaa gaaagaaata gaaacttcag gcagaacatc	850
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gcagttggtt tttgaatgtc tttcactcct tttattggtt aaactccttt	1000
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<210> SEQ ID NO 30
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: Residues 208-216 of the PUMP-1 protein

<400> SEQUENCE: 30

Phe Leu Tyr Ala Ala Thr His Glu Leu
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<210> SEQ ID NO 31
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: Residues 134-142 of the PUMP-1 protein

<400> SEQUENCE: 31

Asn Met Trp Gly Lys Glu Ile Pro Leu
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<210> SEQ ID NO 32
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: Residues 81-89 of the PUMP-1 protein

<400> SEQUENCE: 32

Ile Met Gln Lys Pro Arg Cys Gly Val
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<210> SEQ ID NO 33
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 10-18 of the PUMP-1 protein

<400> SEQUENCE: 33

Cys Leu Leu Pro Gly Ser Leu Ala Leu
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<210> SEQ ID NO 34
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 60-68 of the PUMP-1 protein

<400> SEQUENCE: 34

Lys Glu Met Gln Lys Phe Phe Gly Leu
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<210> SEQ ID NO 35
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 203-211 of the PUMP-1 protein

<400> SEQUENCE: 35

Ser Leu Gly Ile Asn Phe Leu Tyr Ala
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<210> SEQ ID NO 36
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 73-81 of the PUMP-1 protein

<400> SEQUENCE: 36

Met Leu Asn Ser Arg Val Ile Glu Ile
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<210> SEQ ID NO 37
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 4-12 of the PUMP-1 protein

<400> SEQUENCE: 37

Thr Val Leu Cys Ala Val Cys Leu Leu
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<210> SEQ ID NO 38
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 132-140 of the PUMP-1 protein

<400> SEQUENCE: 38

Ala Leu Asn Met Trp Gly Lys Glu Ile
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<210> SEQ ID NO 39
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 109-117 of the PUMP-1 protein

<400> SEQUENCE: 39

Val Thr Tyr Arg Ile Val Ser Tyr Thr
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<210> SEQ ID NO 40
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 127-135 of the PUMP-1 protein

<400> SEQUENCE: 40

Arg Leu Val Ser Lys Ala Leu Asn Met
5

<210> SEQ ID NO 41
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 154-162 of the PUMP-1 protein

<400> SEQUENCE: 41

Ile Met Ile Gly Phe Ala Arg Gly Ala
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<210> SEQ ID NO 42
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 43-51 of the PUMP-1 protein

<400> SEQUENCE: 42

Tyr Leu Tyr Asp Ser Glu Thr Lys Asn
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<210> SEQ ID NO 43
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 140-148 of the PUMP-1 protein

<400> SEQUENCE: 43

Ile Pro Leu His Phe Arg Lys Val Val
5

<210> SEQ ID NO 44
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 146-154 of the PUMP-1 protein

<400> SEQUENCE: 44

Lys Val Val Trp Gly Thr Ala Asp Ile
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<210> SEQ ID NO 45
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 36-44 of the PUMP-1 protein

<400> SEQUENCE: 45

Gln Asp Tyr Leu Lys Arg Phe Tyr Leu
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<210> SEQ ID NO 46
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 2-10 of the PUMP-1 protein

<400> SEQUENCE: 46

Arg Leu Thr Val Leu Cys Ala Val Cys
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<210> SEQ ID NO 47
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 201-209 of the PUMP-1 protein

<400> SEQUENCE: 47

Gly Ser Ser Leu Gly Ile Asn Phe Leu
5

<210> SEQ ID NO 48
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 70-78 of the PUMP-1 protein

<400> SEQUENCE: 48

Ile Thr Gly Met Leu Asn Ser Arg Val
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<210> SEQ ID NO 49
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 205-213 of the PUMP-1 protein

<400> SEQUENCE: 49

Gly Ile Asn Phe Leu Tyr Ala Ala Thr
5

<210> SEQ ID NO 50
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 4-12 of the PUMP-1 protein

<400> SEQUENCE: 50

Thr Val Leu Cys Ala Val Cys Leu Leu
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<210> SEQ ID NO 51

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<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 208-216 of the PUMP-1 protein

<400> SEQUENCE: 51

Phe Leu Tyr Ala Ala Thr His Glu Leu
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<210> SEQ ID NO 52
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 60-68 of the PUMP-1 protein

<400> SEQUENCE: 52

Lys Glu Met Gln Lys Phe Phe Gly Leu
5

<210> SEQ ID NO 53
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 10-18 of the PUMP-1 protein

<400> SEQUENCE: 53

Cys Leu Leu Pro Gly Ser Leu Ala Leu
5

<210> SEQ ID NO 54
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 134-142 of the PUMP-1 protein

<400> SEQUENCE: 54

Asn Met Trp Gly Lys Glu Ile Pro Leu
5

<210> SEQ ID NO 55
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 8-16 of the PUMP-1 protein

<400> SEQUENCE: 55

Ala Val Cys Leu Leu Pro Gly Ser Leu
5

<210> SEQ ID NO 56
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 146-154 of the PUMP-1 protein

<400> SEQUENCE: 56

Lys Val Val Trp Gly Thr Ala Asp Ile
5

<210> SEQ ID NO 57
<211> LENGTH: 9

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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 73-81 of the PUMP-1 protein

<400> SEQUENCE: 57

Met Leu Asn Ser Arg Val Ile Glu Ile
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<210> SEQ ID NO 58
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 81-89 of the PUMP-1 protein

<400> SEQUENCE: 58

Ile Met Gln Lys Pro Arg Cys Gly Val
5

<210> SEQ ID NO 59
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 66-74 of the PUMP-1 protein

<400> SEQUENCE: 59

Phe Gly Leu Pro Ile Thr Gly Met Leu
5

<210> SEQ ID NO 60
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 147-155 of the PUMP-1 protein

<400> SEQUENCE: 60

Val Val Trp Gly Thr Ala Asp Ile Met
5

<210> SEQ ID NO 61
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 212-220 of the PUMP-1 protein

<400> SEQUENCE: 61

Ala Thr His Glu Leu Gly His Ser Leu
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<210> SEQ ID NO 62
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 178-186 of the PUMP-1 protein

<400> SEQUENCE: 62

His Ala Phe Ala Pro Gly Thr Gly Leu
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<210> SEQ ID NO 63
<211> LENGTH: 9
<212> TYPE: PRT

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<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 205-213 of the PUMP-1 protein

<400> SEQUENCE: 63

Gly Ile Asn Phe Leu Tyr Ala Ala Thr
5

<210> SEQ ID NO 64
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 22-30 of the PUMP-1 protein

<400> SEQUENCE: 64

Gln Glu Ala Gly Gly Met Ser Glu Leu
5

<210> SEQ ID NO 65
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 112-120 of the PUMP-1 protein

<400> SEQUENCE: 65

Arg Ile Val Ser Tyr Thr Arg Asp Leu
5

<210> SEQ ID NO 66
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 167-175 of the PUMP-1 protein

<400> SEQUENCE: 66

Tyr Pro Phe Asp Gly Pro Gly Asn Thr
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<210> SEQ ID NO 67
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 3-11 of the PUMP-1 protein

<400> SEQUENCE: 67

Leu Thr Val Leu Cys Ala Val Cys Leu
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<210> SEQ ID NO 68
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 140-148 of the PUMP-1 protein

<400> SEQUENCE: 68

Ile Pro Leu His Phe Arg Lys Val Val
5

<210> SEQ ID NO 69
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<220> FEATURE:
<223> OTHER INFORMATION: Residues 109-117 of the PUMP-1 protein

<400> SEQUENCE: 69

Val Thr Tyr Arg Ile Val Ser Tyr Thr
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<210> SEQ ID NO 70
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 225-233 of the PUMP-1 protein

<400> SEQUENCE: 70

Ser Ser Asp Pro Asn Ala Val Met Tyr
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<210> SEQ ID NO 71
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 78-86 of the PUMP-1 protein

<400> SEQUENCE: 71

Val Ile Glu Ile Met Gln Lys Pro Arg
5

<210> SEQ ID NO 72
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 198-206 of the PUMP-1 protein

<400> SEQUENCE: 72

Trp Thr Asp Gly Ser Ser Leu Gly Ile
5

<210> SEQ ID NO 73
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 238-246 of the PUMP-1 protein

<400> SEQUENCE: 73

Asn Gly Asp Pro Gln Asn Phe Lys Leu
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<210> SEQ ID NO 74
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 92-100 of the PUMP-1 protein

<400> SEQUENCE: 74

Val Ala Glu Tyr Ser Leu Phe Pro Asn
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<210> SEQ ID NO 75
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:

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<223> OTHER INFORMATION: Residues 35-43 of the PUMP-1 protein

<400> SEQUENCE: 75

Ala Gln Asp Tyr Leu Lys Arg Phe Tyr
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<210> SEQ ID NO 76

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 202-210 of the PUMP-1 protein

<400> SEQUENCE: 76

Ser Ser Leu Gly Ile Asn Phe Leu Tyr
5

<210> SEQ ID NO 77

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 46-54 of the PUMP-1 protein

<400> SEQUENCE: 77

Asp Ser Glu Thr Lys Asn Ala Asn Ser
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<210> SEQ ID NO 78

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 87-95 of the PUMP-1 protein

<400> SEQUENCE: 78

Cys Gly Val Pro Asp Val Ala Glu Tyr
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<210> SEQ ID NO 79

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 248-256 of the PUMP-1 protein

<400> SEQUENCE: 79

Gln Asp Asp Ile Lys Gly Ile Gln Lys
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<210> SEQ ID NO 80

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 27-35 of the PUMP-1 protein

<400> SEQUENCE: 80

Met Ser Glu Leu Gln Trp Glu Gln Ala
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<210> SEQ ID NO 81

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 150-158 of the PUMP-1 protein

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<400> SEQUENCE: 81

Gly Thr Ala Asp Ile Met Ile Gly Phe
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<210> SEQ ID NO 82

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 123-131 of the PUMP-1 protein

<400> SEQUENCE: 82

Ile Thr Val Asp Arg Leu Val Ser Lys
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<210> SEQ ID NO 83

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 108-116 of the PUMP-1 protein

<400> SEQUENCE: 83

Val Val Thr Tyr Arg Ile Val Ser Tyr
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<210> SEQ ID NO 84

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 54-62 of the PUMP-1 protein

<400> SEQUENCE: 84

Ser Leu Glu Ala Lys Leu Lys Glu Met
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<210> SEQ ID NO 85

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 163-171 of the PUMP-1 protein

<400> SEQUENCE: 85

His Gly Asp Ser Tyr Pro Phe Asp Gly
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<210> SEQ ID NO 86

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 10-18 of the PUMP-1 protein

<400> SEQUENCE: 86

Cys Leu Leu Pro Gly Ser Leu Ala Leu
5

<210> SEQ ID NO 87

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 151-159 of the PUMP-1 protein

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<400> SEQUENCE: 87

Thr Ala Asp Ile Met Ile Gly Phe Ala
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<210> SEQ ID NO 88

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 138-146 of the PUMP-1 protein

<400> SEQUENCE: 88

Lys Glu Ile Pro Leu His Phe Arg Lys
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<210> SEQ ID NO 89

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 137-145 of the PUMP-1 protein

<400> SEQUENCE: 89

Gly Lys Glu Ile Pro Leu His Phe Arg
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<210> SEQ ID NO 90

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 115-123 of the PUMP-1 protein

<400> SEQUENCE: 90

Ser Tyr Thr Arg Asp Leu Pro His Ile
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<210> SEQ ID NO 91

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 112-120 of the PUMP-1 protein

<400> SEQUENCE: 91

Arg Ile Val Ser Tyr Thr Arg Asp Leu
5

<210> SEQ ID NO 92

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 66-74 of the PUMP-1 protein

<400> SEQUENCE: 92

Phe Gly Leu Pro Ile Thr Gly Met Leu
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<210> SEQ ID NO 93

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 37-45 of the PUMP-1 protein

<400> SEQUENCE: 93

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Asp Tyr Leu Lys Arg Phe Tyr Leu Tyr
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<210> SEQ ID NO 94
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 51-59 of the PUMP-1 protein

<400> SEQUENCE: 94

Asn Ala Asn Ser Leu Glu Ala Lys Leu
5

<210> SEQ ID NO 95
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 166-174 of the PUMP-1 protein

<400> SEQUENCE: 95

Ser Tyr Pro Phe Lys Gly Pro Gly Asn
5

<210> SEQ ID NO 96
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 10-18 of the PUMP-1 protein

<400> SEQUENCE: 96

Cys Leu Leu Pro Gly Ser Leu Ala Leu
5

<210> SEQ ID NO 97
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 4-12 of the PUMP-1 protein

<400> SEQUENCE: 97

Thr Val Leu Cys Ala Val Cys Leu Leu
5

<210> SEQ ID NO 98
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 31-39 of the PUMP-1 protein

<400> SEQUENCE: 98

Gln Trp Glu Gln Ala Gln Asp Tyr Leu
5

<210> SEQ ID NO 99
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 3-11 of the PUMP-1 protein

<400> SEQUENCE: 99

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Leu Thr Val Leu Cys Ala Val Cys Leu
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<210> SEQ ID NO 100
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 212-220 of the PUMP-1 protein

<400> SEQUENCE: 100

Ala Thr His Glu Leu Gly His Ser Leu
5

<210> SEQ ID NO 101
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 238-246 of the PUMP-1 protein

<400> SEQUENCE: 101

Asn Gly Asp Pro Gln Asn Phe Lys Leu
5

<210> SEQ ID NO 102
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 44-52 of the PUMP-1 protein

<400> SEQUENCE: 102

Leu Tyr Asp Ser Glu Thr Lys Asn Ala
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<210> SEQ ID NO 103
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 235-243 of the PUMP-1 protein

<400> SEQUENCE: 103

Thr Tyr Gly Asn Gly Asp Pro Gln Asn
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<210> SEQ ID NO 104
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 243-251 of the PUMP-1 protein

<400> SEQUENCE: 104

Asn Phe Lys Leu Ser Gln Asp Asp Ile
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<210> SEQ ID NO 105
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 8-16 of the PUMP-1 protein

<400> SEQUENCE: 105

Ala Val Cys Leu Leu Pro Gly Ser Leu

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<210> SEQ ID NO 106
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 12-20 of the PUMP-1 protein

<400> SEQUENCE: 106

Leu Pro Gly Ser Leu Ala Leu Pro Leu
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<210> SEQ ID NO 107
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 58-66 of the PUMP-1 protein

<400> SEQUENCE: 107

Lys Leu Lys Glu Met Gln Lys Phe Phe
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<210> SEQ ID NO 108
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 201-209 of the PUMP-1 protein

<400> SEQUENCE: 108

Gly Ser Ser Leu Gly Ile Asn Phe Leu
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<210> SEQ ID NO 109
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 208-216 of the PUMP-1 protein

<400> SEQUENCE: 109

Phe Leu Tyr Ala Ala Thr His Glu Leu
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<210> SEQ ID NO 110
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 120-128 of the PUMP-1 protein

<400> SEQUENCE: 110

Leu Pro His Ile Thr Val Asp Arg Leu
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<210> SEQ ID NO 111
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 12-20 of the PUMP-1 protein

<400> SEQUENCE: 111

Leu Pro Gly Ser Leu Ala Leu Pro Leu
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<210> SEQ ID NO 112
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 8-16 of the PUMP-1 protein

<400> SEQUENCE: 112

Ala Val Cys Leu Leu Pro Gly Ser Leu
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<210> SEQ ID NO 113
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 84-92 of the PUMP-1 protein

<400> SEQUENCE: 113

Lys Pro Arg Cys Gly Val Pro Asp Val
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<210> SEQ ID NO 114
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 89-97 of the PUMP-1 protein

<400> SEQUENCE: 114

Val Pro Asp Val Ala Glu Tyr Ser Leu
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<210> SEQ ID NO 115
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 4-12 of the PUMP-1 protein

<400> SEQUENCE: 115

Thr Val Leu Cys Ala Val Cys Leu Leu
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<210> SEQ ID NO 116
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 178-186 of the PUMP-1 protein

<400> SEQUENCE: 116

His Ala Phe Ala Pro Gly Thr Gly Leu
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<210> SEQ ID NO 117
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 51-59 of the PUMP-1 protein

<400> SEQUENCE: 117

Asn Ala Asn Ser Leu Glu Ala Lys Leu
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<210> SEQ ID NO 118
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 212-220 of the PUMP-1 protein

<400> SEQUENCE: 118

Ala Thr His Glu Leu Gly His Ser Leu
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<210> SEQ ID NO 119
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 140-148 of the PUMP-1 protein

<400> SEQUENCE: 119

Ile Pro Leu His Phe Arg Lys Val Val
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<210> SEQ ID NO 120
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 147-155 of the PUMP-1 protein

<400> SEQUENCE: 120

Val Val Trp Gly Thr Ala Asp Ile Met
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<210> SEQ ID NO 121
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 208-216 of the PUMP-1 protein

<400> SEQUENCE: 121

Phe Leu Tyr Ala Ala Thr His Glu Leu
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<210> SEQ ID NO 122
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 101-109 of the PUMP-1 protein

<400> SEQUENCE: 122

Ser Pro Lys Trp Thr Ser Lys Val Val
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<210> SEQ ID NO 123
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 10-18 of the PUMP-1 protein

<400> SEQUENCE: 123

Cys Leu Leu Pro Gly Ser Leu Ala Leu
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<210> SEQ ID NO 124
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 3-11 of the PUMP-1 protein

<400> SEQUENCE: 124

Leu Thr Val Leu Cys Ala Val Cys Leu
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<210> SEQ ID NO 125
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 201-209 of the PUMP-1 protein

<400> SEQUENCE: 125

Gly Ser Ser Leu Gly Ile Asn Phe Leu
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<210> SEQ ID NO 126
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 134-142 of the PUMP-1 protein

<400> SEQUENCE: 126

Asn Met Trp Gly Lys Glu Ile Pro Leu
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<210> SEQ ID NO 127
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 112-120 of the PUMP-1 protein

<400> SEQUENCE: 127

Arg Ile Val Ser Tyr Thr Arg Asp Leu
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<210> SEQ ID NO 128
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 125-133 of the PUMP-1 protein

<400> SEQUENCE: 128

Val Asp Arg Leu Val Ser Lys Ala Leu
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<210> SEQ ID NO 129
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 66-74 of the PUMP-1 protein

<400> SEQUENCE: 129

Phe Gly Leu Pro Ile Thr Gly Met Leu
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<210> SEQ ID NO 130

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<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 134-142 of the PUMP-1 protein

<400> SEQUENCE: 130

Asn Met Trp Gly Lys Glu Ile Pro Leu
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<210> SEQ ID NO 131
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 56-64 of the PUMP-1 protein

<400> SEQUENCE: 131

Glu Ala Lys Leu Lys Glu Met Gln Lys
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<210> SEQ ID NO 132
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 101-109 of the PUMP-1 protein

<400> SEQUENCE: 132

Ser Pro Lys Trp Thr Ser Lys Val Val
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<210> SEQ ID NO 133
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 73-81 of the PUMP-1 protein

<400> SEQUENCE: 133

Met Leu Asn Ser Arg Val Ile Glu Ile
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<210> SEQ ID NO 134
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 84-92 of the PUMP-1 protein

<400> SEQUENCE: 134

Lys Pro Arg Cys Gly Val Pro Asp Val
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<210> SEQ ID NO 135
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 127-135 of the PUMP-1 protein

<400> SEQUENCE: 135

Arg Leu Val Ser Lys Ala Leu Asn Met
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<210> SEQ ID NO 136
<211> LENGTH: 9

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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 105-113 of the PUMP-1 protein

<400> SEQUENCE: 136

Thr Ser Lys Val Val Thr Tyr Arg Ile
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<210> SEQ ID NO 137
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 51-59 of the PUMP-1 protein

<400> SEQUENCE: 137

Asn Ala Asn Ser Leu Glu Ala Lys Leu
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<210> SEQ ID NO 138
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 12-20 of the PUMP-1 protein

<400> SEQUENCE: 138

Leu Pro Gly Ser Leu Ala Leu Pro Leu
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<210> SEQ ID NO 139
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 120-128 of the PUMP-1 protein

<400> SEQUENCE: 139

Leu Pro His Ile Thr Val Asp Arg Leu
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<210> SEQ ID NO 140
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 178-186 of the PUMP-1 protein

<400> SEQUENCE: 140

His Ala Phe Ala Pro Gly Thr Gly Leu
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<210> SEQ ID NO 141
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 54-62 of the PUMP-1 protein

<400> SEQUENCE: 141

Ser Leu Glu Ala Lys Leu Lys Glu Met
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<210> SEQ ID NO 142
<211> LENGTH: 9
<212> TYPE: PRT

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<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 10-18 of the PUMP-1 protein

<400> SEQUENCE: 142

Cys Leu Leu Pro Gly Ser Leu Ala Leu
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<210> SEQ ID NO 143
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 208-216 of the PUMP-1 protein

<400> SEQUENCE: 143

Phe Leu Tyr Ala Ala Thr His Glu Leu
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<210> SEQ ID NO 144
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 125-133 of the PUMP-1 protein

<400> SEQUENCE: 144

Val Asp Arg Leu Val Ser Lys Ala Leu
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<210> SEQ ID NO 145
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 158-166 of the PUMP-1 protein

<400> SEQUENCE: 145

Phe Ala Arg Gly Ala His Gly Asp Ser
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<210> SEQ ID NO 146
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 36-44 of the PUMP-1 protein

<400> SEQUENCE: 146

Gln Asp Tyr Leu Lys Arg Phe Tyr Leu
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<210> SEQ ID NO 147
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 212-220 of the PUMP-1 protein

<400> SEQUENCE: 147

Ala Thr His Glu Leu Gly His Ser Leu
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<210> SEQ ID NO 148
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<220> FEATURE:
<223> OTHER INFORMATION: Residues 116-124 of the PUMP-1 protein

<400> SEQUENCE: 148

Tyr Thr Arg Asp Leu Pro His Ile Thr
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<210> SEQ ID NO 149
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 62-70 of the PUMP-1 protein

<400> SEQUENCE: 149

Met Gln Lys Phe Phe Gly Leu Pro Ile
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<210> SEQ ID NO 150
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 159-167 of the PUMP-1 protein

<400> SEQUENCE: 150

Ala Thr Gly Ala His Gly Asp Ser Tyr
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<210> SEQ ID NO 151
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 30-38 of the PUMP-1 protein

<400> SEQUENCE: 151

Leu Gln Trp Glu Gln Ala Gln Asp Tyr
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<210> SEQ ID NO 152
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 196-204 of the PUMP-1 protein

<400> SEQUENCE: 152

Glu Arg Trp Thr Asp Gly Ser Ser Leu
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<210> SEQ ID NO 153
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 40-48 of the PUMP-1 protein

<400> SEQUENCE: 153

Lys Arg Phe Tyr Leu Tyr Asp Ser Glu
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<210> SEQ ID NO 154
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:

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<223> OTHER INFORMATION: Residues 1-9 of the PUMP-1 protein

<400> SEQUENCE: 154

Met Arg Leu Thr Val Leu Cys Ala Val
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<210> SEQ ID NO 155

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 144-152 of the PUMP-1 protein

<400> SEQUENCE: 155

Phe Arg Lys Val Val Trp Gly Thr Ala
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<210> SEQ ID NO 156

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 117-125 of the PUMP-1 protein

<400> SEQUENCE: 156

Thr Arg Asp Leu Pro His Ile Thr Val
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<210> SEQ ID NO 157

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 134-142 of the PUMP-1 protein

<400> SEQUENCE: 157

Asn Met Trp Gly Lys Glu Ile Pro Leu
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<210> SEQ ID NO 158

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 96-104 of the PUMP-1 protein

<400> SEQUENCE: 158

Ser Leu Phe Pro Asn Ser Pro Lys Trp
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<210> SEQ ID NO 159

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 62-70 of the PUMP-1 protein

<400> SEQUENCE: 159

Met Gln Lys Phe Phe Gly Leu Pro Ile
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<210> SEQ ID NO 160

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 35-43 of the PUMP-1 protein

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<400> SEQUENCE: 160

Ala Gln Asp Tyr Leu Lys Arg Phe Tyr
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<210> SEQ ID NO 161

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 208-216 of the PUMP-1 protein

<400> SEQUENCE: 161

Phe Leu Tyr Ala Ala Thr His Glu Leu
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<210> SEQ ID NO 162

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 76-84 of the PUMP-1 protein

<400> SEQUENCE: 162

Ser Arg Val Ile Glu Ile Met Gln Lys
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<210> SEQ ID NO 163

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 126-134 of the PUMP-1 protein

<400> SEQUENCE: 163

Asp Arg Leu Val Ser Lys Ala Leu Asn
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<210> SEQ ID NO 164

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 60-68 of the PUMP-1 protein

<400> SEQUENCE: 164

Lys Glu Met Gln Lys Phe Phe Gly Leu
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<210> SEQ ID NO 165

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 58-66 of the PUMP-1 protein

<400> SEQUENCE: 165

Lys Leu Lys Glu Met Gln Lys Phe Phe
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<210> SEQ ID NO 166

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 256-264 of the PUMP-1 protein

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<400> SEQUENCE: 166

Lys Leu Tyr Gly Lys Arg Ser Asn Ser
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<210> SEQ ID NO 167

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 85-93 of the PUMP-1 protein

<400> SEQUENCE: 167

Pro Arg Cys Gly Val Pro Asp Val Ala
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<210> SEQ ID NO 168

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 111-119 of the PUMP-1 protein

<400> SEQUENCE: 168

Tyr Arg Ile Val Ser Tyr Thr Arg Asp
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<210> SEQ ID NO 169

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 178-186 of the PUMP-1 protein

<400> SEQUENCE: 169

His Ala Phe Ala Pro Gly Thr Gly Leu
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<210> SEQ ID NO 170

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 87-95 of the PUMP-1 protein

<400> SEQUENCE: 170

Cys Gly Val Pro Asp Val Ala Glu Tyr
5

<210> SEQ ID NO 171

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 202-210 of the PUMP-1 protein

<400> SEQUENCE: 171

Ser Ser Leu Gly Ile Asn Phe Leu Tyr
5

<210> SEQ ID NO 172

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 79-87 of the PUMP-1 protein

<400> SEQUENCE: 172

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Ile Glu Ile Met Gln Lys Pro Arg Cys
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<210> SEQ ID NO 173
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 60-68 of the PUMP-1 protein

<400> SEQUENCE: 173

Lys Glu Met Gln Lys Phe Phe Gly Leu
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<210> SEQ ID NO 174
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 225-233 of the PUMP-1 protein

<400> SEQUENCE: 174

Ser Ser Asp Pro Asn Ala Val Met Tyr
5

<210> SEQ ID NO 175
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 47-55 of the PUMP-1 protein

<400> SEQUENCE: 175

Ser Glu Thr Lys Asn Ala Asn Ser Leu
5

<210> SEQ ID NO 176
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 195-203 of the PUMP-1 protein

<400> SEQUENCE: 176

Asp Glu Arg Trp Thr Asp Gly Ser Ser
5

<210> SEQ ID NO 177
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 214-222 of the PUMP-1 protein

<400> SEQUENCE: 177

His Glu Leu Gly His Ser Leu Gly Met
5

<210> SEQ ID NO 178
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 22-30 of the PUMP-1 protein

<400> SEQUENCE: 178

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Gln Glu Ala Gly Gly Met Ser Glu Leu
5

<210> SEQ ID NO 179
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 249-257 of the PUMP-1 protein

<400> SEQUENCE: 179

Asp Asp Ile Lys Gly Ile Gln Lys Leu
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<210> SEQ ID NO 180
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 93-101 of the PUMP-1 protein

<400> SEQUENCE: 180

Ala Glu Tyr Ser Leu Phe Pro Asn Ser
5

<210> SEQ ID NO 181
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 138-146 of the PUMP-1 protein

<400> SEQUENCE: 181

Lys Glu Ile Pro Leu His Phe Arg Lys

<210> SEQ ID NO 182
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 184-192 of the PUMP-1 protein

<400> SEQUENCE: 182

Thr Gly Leu Gly Gly Asp Ala His Phe
5

<210> SEQ ID NO 183
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 200-208 of the PUMP-1 protein

<400> SEQUENCE: 183

Asp Gly Ser Ser Leu Gly Ile Asn Phe
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<210> SEQ ID NO 184
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 35-43 of the PUMP-1 protein

<400> SEQUENCE: 184

Ala Gln Asp Tyr Leu Lys Arg Phe Tyr
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<210> SEQ ID NO 185
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
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 <223> OTHER INFORMATION: Residues 34-42 of the PUMP-1 protein

<400> SEQUENCE: 185

Gln Ala Gln Asp Tyr Leu Lys Arg Phe
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<210> SEQ ID NO 186
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
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<400> SEQUENCE: 186

Leu Gln Trp Glu Gln Ala Gln Asp Tyr
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<210> SEQ ID NO 187
 <211> LENGTH: 9
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 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: Residues 250-258 of the PUMP-1 protein

<400> SEQUENCE: 187

Asp Ile Lys Gly Ile Gln Lys Leu Tyr
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<210> SEQ ID NO 188
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 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: Residues 150-158 of the PUMP-1 protein

<400> SEQUENCE: 188

Gly Thr Ala Asp Ile Met Ile Gly Phe

<210> SEQ ID NO 189
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 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: Residues 37-45 of the PUMP-1 protein

<400> SEQUENCE: 189

Asp Tyr Leu Lys Arg Phe Tyr Leu Tyr
 1 5

What is claimed is:

1. A method of diagnosing ovarian, breast or lung cancer in an individual, comprising the steps of:

- (a) obtaining a biological sample from an individual; and
- (b) detecting PUMP-1 in said sample, wherein over-expression of PUMP-1 in said sample compared to a sample of normal tissue is indicative of the presence of ovarian, breast or lung cancer in said individual.

2. The method of claim 1, wherein said biological sample is selected from the group consisting of blood, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

3. The method of claim 1, wherein said detection of said PUMP-1 is by means selected from the group consisting of Western blot, dot blot, ELISA sandwich assay, radioimmunoassay, and flow cytometry.

4. A method for detecting malignant ovarian, breast or lung hyperplasia in a biological sample, comprising the steps of:

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- (a) isolating protein from said sample; and
- (b) detecting PUMP-1 protein in said sample, wherein over-expression of said PUMP-1 protein in said sample compared to a sample of normal tissue is indicative of the presence of malignant ovarian, breast or lung hyper-
plasia. 5

5. The method of claim 4, wherein said detection is by immunoaffinity to an antibody, wherein said antibody is specific for PUMP-1.

6. The method of claim 4, wherein said biological sample is selected from the group consisting of blood, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells. 10

7. A method for detecting ovarian malignant hyperplasia in a biological sample, comprising the steps of:

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- (a) isolating proteases present in said biological sample; and

- (b) detecting specific proteases selected from the group consisting of hepsin, SCCE, and PUMP-1 present in said biological sample, wherein over-expression of said proteases in said sample compared to normal ovarian tissue sample is indicative of the presence of malignant ovarian hyperplasia.

8. The method of claim 7, wherein said protease is detected with an antibody.

9. The method of claim 7, wherein said biological sample is selected from the group consisting of blood, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

* * * * *

专利名称(译)	早期诊断卵巢癌，乳腺癌和肺癌的方法		
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当前申请(专利权)人(译)	生物仿制药，LLC		
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摘要(译)

所公开的核酸引物组与组织cDNA的定量扩增 (PCR) 组合使用，可以指示组织样品中特定蛋白酶的存在。检测到的蛋白酶本身在某些癌症中特异性过表达，并且它们的存在可用于相关卵巢和其他恶性肿瘤的早期检测，以及用于癌症治疗的相互作用疗法的设计。

