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(54) Title: COMPOSITIONS AND METHODS FOR THE EARLY DIAGNOSIS OF OVARIAN CANCER

(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.

COMPOSITIONS AND METHODS FOR THE EARLY DIAGNOSIS OF OVARIAN CANCER

BACKGROUND OF THE INVENTION

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 cancer, especially ovarian cancer diagnosis.

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 improved greatly 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
Ser Proteases:	uPA	uPA	NES-1	NES-1
	PAI-1	PAI-1	uPA	uPA
		tPA		PAI-2
Cys Proteases:	Cathepsin B	Cathepsin L	Cathepsin B	Cathepsin B
	Cathepsin L		Cathepsin L	Cathepsin L
Metal-proteases:	Matrilysin*	Matrilysin	Stromelysin-3	MMP-2
	Collagenase	Stromelysin	MMP-8	
	Stromelysin-l*	Gelatinase B	MMP-9	
			Gelatinase A	

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 tumorgenic, 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 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 stratum corneum chymotrytic enzyme (SCCE) mRNA in tissue, which is indicative of stratum corneum chymotrytic enzyme 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 SCCE or expression of SCCE), 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 obtaining a biological sample from the individual and detecting stratum corneum chymotrytic enzyme in the sample. Usually, the presence of stratum corneum chymotrytic enzyme in the sample is indicative of the presence of carcinoma in the individual, and the absence of stratum corneum chymotrytic enzyme in the sample is indicative of the absence of carcinoma in the individual.

In another embodiment of the present invention, there is provided a method for detecting malignant hyperplasia in a biological sample, comprising the steps of isolating mRNA from the sample and detecting stratum corneum chymotrytic enzyme mRNA in the sample. Typically, the presence of the stratum corneum chymotrytic enzyme mRNA in the sample is indicative of the presence of malignant hyperplasia, and the absence of the SCCE mRNA in the sample is indicative of the absence of malignant hyperplasia.

In yet another embodiment of the present invention, there is provided a method for detecting malignant hyperplasia in a biological sample, comprising the steps of isolating protein from the sample and detecting stratum corneum chymotrytic enzyme protein in the sample. Generally, the presence of the SCCE protein in the sample is indicative of the presence of malignant hyperplasia, wherein the absence of the SCCE protein in the sample is indicative of the absence of malignant hyperplasia. This method may further comprise the step of comparing the SCCE protein to reference information, wherein the comparison provides a diagnosis of the malignant hyperplasia, or alternatively, determines a treatment of the malignant hyperplasia.

In still yet another embodiment of the present invention,

there is provided a method of inhibiting expression of stratum corneum chymotrytic enzyme in a cell, comprising the step of introducing a vector into the cell, wherein the vector comprises a stratum corneum chymotrytic enzyme gene in opposite orientation operably linked to elements necessary for expression. Expression of the vector produces SCCE antisense mRNA in the cell, which hybridizes to endogenous SCCE mRNA and thereby inhibits expression of SCCE in the cell.

In yet another embodiment of the present invention, there is provided a method of inhibiting stratum corneum chymotrytic enzyme protein in a cell, comprising the step of introducing an antibody specific for stratum corneum chymotrytic enzyme protein or a fragment thereof into the cell. Binding of the antibody inhibits the SCCE protein.

In another embodiment of the present invention, there is provided a method of targeted therapy to an individual, comprising the step of administering a compound to an individual, wherein the compound has a targeting moiety and a therapeutic moiety, wherein the targeting moiety is specific for stratum corneum chymotrytic enzyme.

In yet another embodiment of the present invention, there is provided a method of vaccinating an individual against stratum corneum chymotrytic enzyme, comprising the steps of inoculating an individual with the stratum corneum chymotrytic enzyme protein or fragment thereof, wherein the stratum corneum chymotrytic enzyme protein or fragment thereof lacks SCCE protease activity. Inoculation with the stratum corneum chymotrytic enzyme protein or fragment thereof elicits an immune response in the individual, thereby

vaccinating the individual against stratum corneum chymotrytic enzyme.

In still another embodiment of the present invention, there is provided an oligonucleotide having a sequence complementary to SEQ ID No. 30 (i.e., full length nucleotide sequence of SCCE, or fragments thereof as would be readily recognizable to one having ordinary skill in this art). Also embodied is a composition comprising the above-described oligonucleotide and a physiologically acceptable carrier therefore. Additionally embodied is a method of treating a neoplastic state in an individual in need of such treatment, comprising the step of administering to the individual an effective dose of the above-described oligonucleotide.

In another embodiment of the present invention, there is provided a method of screening for compounds that inhibit stratum corneum chymotrytic enzyme activity, comprising the steps of contacting a sample with a compound, wherein the sample comprises SCCE protein; and assaying for SCCE protease activity. A decrease in the SCCE protease activity in the presence of the compound relative to SCCE protease activity in the absence of the compound is indicative of a compound that inhibits stratum corneum chymotrytic enzyme activity.

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.

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

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

Figure 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).

Figure 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).

Figure 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).

Figure 6 shows quantitative PCR analysis of SCCE expression. Cases 3, 4 and 9 are normal ovaries. Cases 19, 21, 14, 15 and 16 are LMP tumors. Cases 43, 23, 36 and 37 are ovarian

carcinomas. Expression levels of stratum corneum chymotrytic enzyme relative to β-tubulin are significantly elevated in tumor Cases 19, 14, 15, 16, 43, 23, 36 and 37 compared to that of normal ovaries.

Figure 7A shows Northern blot analysis of stratum corneum chymotrytic enzyme mRNA from normal ovary and ovarian carcinomas. Lane 1, normal ovary (case 10); Lane 2, serous carcinoma (case 35); Lane 3, mucinous carcinoma (case 48); Lane 4, endometrioid carcinoma (case 51); and Lane 5, clear cell carcinoma (case 54). Two transcripts (1.2 and 2.0 kb) were detected in all of the subtypes of carcinoma (lanes 2-5). **Figures 7B** and **7C** show that normal human adult tissues (spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas) and normal human fetal tissues (brain, lung, liver and kidney) examined showed no visible SCCE transcripts.

Figure 8 shows the ratio of SCCE expression to expression of β-tubulin in normal ovary, LMP tumor and ovarian carcinoma. SCCE mRNA expression levels were significantly elevated in LMP tumor ($p<0.05$) and carcinoma ($p<0.001$) compared to that in normal ovary. All 10 cases of normal ovaries showed a low level of SCCE mRNA expression.

Figure 9 shows MDA-MB-435S (Lanes 1 & 3) and HeLa (Lanes 2 & 4) cell lysates were separated by SDS-PAGE and immunoblotted. Lanes 1 & 2 were probed with rabbit pre-immune serum as a negative control. Lanes 3 & 4 were probed with polyclonal rabbit antibodies generated to peptides derived from SCCE protein sequence.

Figure 10A shows normal surface ovarian epithelium.

Little SCCE expression was observed (normal ovary, X100). **Figure 10B** is a negative control section for Figure 10A. No nonspecific staining was observed (Normal ovary, X100). **Figure 10C** shows positive SCCE staining localized in the cytoplasm and the cell membrane of ovarian cancer cells (case 947, clear cell adenocarcinoma, X100). **Figure 10D** is a negative control section for Figure 10C. No nonspecific staining was observed (case 947, clear cell adenocarcinoma, X100). **Figure 10E** is positive stratum corneum chymotrytic enzyme staining localized in the cytoplasm and the cell membrane of ovarian cancer cells. Mucin in the glands also showed positive stratum corneum chymotrytic enzyme staining (case 947, clear cell adenocarcinoma, X100). **Figure 10F** is a negative control section for Figure 10E. No nonspecific staining was observed (case 947, clear cell adenocarcinoma, X100).

Figure 11A shows Northern blot analysis of hepsin expression in normal ovary and ovarian carcinomas. Lane 1, normal ovary (case 10); lane 2, serous carcinoma (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 **(Figure 11B)** and adult tissues **(Figure 11C)**. 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.

Figure 12A shows hepsin expression in normal (N), mucinous (M) and serous (S) low malignant potential (LMP) tumors

and carcinomas (CA). **Figure 12B** shows a bar graph of expression of hepsin in 10 normal ovaries and 44 ovarian carcinoma samples.

Figure 13 shows a comparison by quantitative PCR of normal and ovarian carcinoma expression of mRNA for protease M.

Figure 14 shows the TADG-12 catalytic domain including an insert near the His 5'-end.

Figure 15A shows northern blot analysis comparing TADG-14 expression in normal and ovarian carcinoma tissues. **Figure 15B** shows preliminary quantitative PCR amplification of normal and carcinoma cDNAs using specific primers for TADG-14.

Figure 16A shows northern blot analysis of the PUMP-1 gene in normal ovary and ovarian carcinomas. **Figure 16B** shows northern blot analysis of the PUMP-1 gene in human fetal tissue. **Figure 16C** shows northern blot analysis of the PUMP-1 gene in adult tissues.

Figure 17A shows a comparison of PUMP-1 expression in normal and carcinoma tissues using quantitative PCR with an internal β-tubulin control. **Figure 17B** shows the ratio of mRNA expression of PUMP-1 compared to the internal control β-tubulin in 10 normal and 44 ovarian carcinomas.

Figure 18 shows a comparison of Cathepsin L expression in normal and carcinoma tissues using quantitative PCR with an internal β-tubulin control.

Figure 19 is a summary of PCR amplified products for the hepsin, SCCE, protease M, PUMP-1 and Cathepsin L genes.

DETAILED DESCRIPTION OF THE INVENTION

This invention identifies that the SCCE protease on ovarian and other tumor cells 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 down regulation, 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.

A primary object of the present invention is a method for detecting the presence of malignant hyperplasia in a tissue sample. It is an advantage of the present invention that it has as a particular object the detection of cancer in ovarian tissue. The cancer is detected by analyzing a biological sample for the presence of markers to proteases that are specific indicators of certain types of cancer cells. This object may be accomplished by isolating mRNA from a sample or by detection of proteins by polyclonal or preferably monoclonal antibodies. When using mRNA detection, the method may be carried out by combining the isolated mRNA with reagents to convert to cDNA according to standard methods; treating the converted cDNA with amplification reaction reagents (such as cDNA PCR reaction reagents) in a container along with an appropriate mixture of nucleic acid primers selected from the list in Table 2 or as

detailed above; reacting the contents of the container to produce amplification products; and analyzing the amplification products to detect the presence of malignant hyperplasia markers in the sample. For mRNA, the analyzing step may be accomplished using Northern Blot analysis to detect the presence of malignant hyperplasia markers in the amplification product. Northern Blot analysis is known in the art. The analysis step may be further accomplished by quantitatively detecting the presence of malignant hyperplasia marker in the amplification produce, and comparing the quantity of marker detected against a panel of expected values for known presence or absence in normal and malignant tissue derived using similar primers.

Another embodiment of the present invention are various nucleic acid sequences that are useful in the methods disclosed herein. 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 No. 1 with SEQ ID No. 2; SEQ ID No. 1 with SEQ ID No. 3; SEQ ID No. 4 with SEQ ID No. 5; SEQ ID No. 6 with SEQ ID No. 7; SEQ ID No. 8 with SEQ ID No. 9; and SEQ ID No. 10 with SEQ ID No. 11. 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.

The present invention is directed toward a method of diagnosing cancer in an individual, comprising the steps of obtaining a biological sample from an individual and detecting stratum corneum chymotrytic enzyme in the sample. The presence of SCCE in the sample is indicative of the presence of cancer in the individual,

wherein the absence of SCCE in the sample is indicative of the absence of cancer in the individual. Generally, detection of SCCE is by means such as Northern blot, Western blot, PCR, dot blot, ELISA sandwich assay, radioimmunoassay, DNA array chips and flow cytometry. An example of a typical cancer diagnosed by this method is ovarian cancer.

The present invention is also directed toward a method for detecting malignant hyperplasia in a biological sample, comprising the steps of isolating mRNA from the sample; and detecting SCCE mRNA in the sample. The presence of the SCCE mRNA in the sample is indicative of the presence of malignant hyperplasia, wherein the absence of the SCCE mRNA in the sample is indicative of the absence of malignant hyperplasia. This method may further comprise the step of comparing the SCCE mRNA to reference information, wherein the comparison provides a diagnosis and/or determines a treatment of the malignant hyperplasia. A typical means of detection of stratum corneum chymotrytic enzyme mRNA is by PCR amplification, which, preferably, uses primers shown in SEQ ID No. 10 and SEQ ID No. 11. Representative biological samples include a tissue and a bodily fluid, wherein the bodily fluid is preferably blood.

The present invention is additionally directed toward a method for detecting malignant hyperplasia in a biological sample, comprising the steps of isolating protein from the sample; and detecting stratum corneum chymotrytic enzyme protein in the sample. The presence of stratum corneum chymotrytic enzyme protein in the sample is indicative of the presence of malignant hyperplasia, wherein the absence of SCCE protein in the sample is indicative of the absence of malignant hyperplasia. This method also

may comprise the step of comparing SCCE protein to reference information, wherein the comparison provides a diagnosis or determines a treatment of the malignant hyperplasia. Preferably, the detection of SCCE protein is by immunoaffinity to an antibody which is specific for SCCE. Representative biological samples are a tissue and a bodily fluid, and it is preferable that the bodily fluid is blood.

The present invention is further directed toward a method of inhibiting expression of stratum corneum chymotrytic enzyme in a cell, comprising the step of introducing a vector into a cell, wherein the vector comprises a SCCE gene in opposite orientation operably linked to elements necessary for expression, wherein expression of the vector produces SCCE antisense mRNA in the cell. The SCCE antisense mRNA hybridizes to endogenous SCCE mRNA, thereby inhibiting expression of stratum corneum chymotrytic enzyme in the cell.

The present invention is still further directed toward a method of inhibiting stratum corneum chymotrytic enzyme protein in a cell, comprising the step of introducing an antibody into a cell, wherein the antibody is specific for stratum corneum chymotrytic enzyme protein or a fragment thereof. Binding of the antibody to SCCE inhibits the SCCE protein. Preferably, the stratum corneum chymotrytic enzyme fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 31, 32, 33, 34, 35, 36, 80, 86 or 99.

The present invention is also directed toward a method of targeted therapy to an individual, comprising the step of administering a compound to an individual, wherein the compound has a targeting moiety and a therapeutic moiety, and wherein the

targeting moiety is specific for stratum corneum chymotrytic enzyme. Preferably, the targeting moiety is an antibody specific for SCCE or a ligand or ligand binding domain that binds SCCE. Likewise, the therapeutic moiety is preferably a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant or cytotoxic agent. Generally, the individual suffers from a disease such as ovarian cancer, lung cancer, prostate cancer, colon cancer or another cancer in which SCCE is overexpressed.

The present invention is additionally directed toward a method of vaccinating an individual against stratum corneum chymotrytic enzyme, comprising the steps of inoculating an individual with SCCE protein or fragment thereof, wherein the SCCE protein or fragment thereof lacks SCCE protease activity. Inoculation with the SCCE protein, or fragment thereof, elicits an immune response in the individual, thereby vaccinating the individual against SCCE. Preferably, the stratum corneum chymotrytic enzyme fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 31, 32, 33, 34, 35, 36, 80, 86 or 99. Generally, this method is applicable when the individual has a cancer, is suspected of having a cancer or is at risk of getting a cancer.

The present invention is yet directed toward a method of producing immune-activated cells directed toward stratum corneum chymotrytic enzyme, comprising the steps of exposing dendritic cells to a SCCE protein or fragment thereof, which lacks SCCE protease activity. Typically, exposure to the SCCE protein or fragment thereof activates the dendritic cells, thereby producing immune-activated cells directed toward stratum corneum chymotrytic enzyme.

Generally, the immune-activated cells are B-cells, T-cells and/or dendrites. Preferably, the SCCE fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 31, 32, 33, 34, 35, 36, 80, 86 or 99. 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 further directed toward an immunogenic composition, comprising an immunogenic fragment of a SCCE 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. 31, 32, 33, 34, 35, 36, 80, 86 or 99.

The present invention is further directed toward an oligonucleotide having a sequence complementary to SEQ ID No. 30 or a fragment thereof. The present invention further provides a composition comprising the above-described oligonucleotide and a physiologically acceptable carrier therefore, and a method of treating a neoplastic state in an individual in need of such treatment, comprising the step of administering to the individual an effective dose of the above-described oligonucleotide. Typically, the neoplastic state may be ovarian cancer, breast cancer, lung cancer, colon cancer, prostate cancer or another cancer in which SCCE is overexpressed.

The present invention is still further directed toward a method of screening for compounds that inhibit stratum corneum chymotrytic enzyme activity, comprising the steps of contacting a

sample with a compound, wherein the sample comprises SCCE protein; and assaying for SCCE protease activity. A decrease in the SCCE protease activity in the presence of the compound relative to SCCE protease activity in the absence of the compound is indicative of a compound that inhibits stratum corneum chymotrytic enzyme activity.

The present invention is yet additionally directed toward a method for detecting ovarian malignant hyperplasia in a biological sample, comprising the steps of isolating the proteases or protease mRNA present in the biological sample; and detecting specific proteases or protease mRNA present in the biological sample. The proteases are selected from the group consisting of hepsin, protease M, complement factor B, SCCE, 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 or determines a treatment of the malignant hyperplasia. Typically, the protease mRNA is detected by amplification of total mRNA, and the protease is detected with an antibody. Representative biological samples are blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

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. Patent 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. NH_2 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.

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 mammalian 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 ^{3}H, ^{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. Patent 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 quantitiy 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. Patent 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 SCCE 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 SCCE protein of the present invention for purposes of prokaryote transformation. Prokaryotic hosts may include *E. coli*, *S. tymphimurium*, *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 an 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. 30 and which encodes an alternative splice variant of stratum corneum chymotrytic enzyme.

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 SCCE protein may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a SCCE 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 SCCE, 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 complementary 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. 30 or the complement thereof. Such a probe is useful for detecting expression of SCCE in a cell by a method including the steps of (a) contacting mRNA obtained from the cell with a labeled SCCE 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 0.1X 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 2X SSC containing 1% SDS; followed by a second wash at about 65°C with about 0.1X SSC.

The DNA may have at least about 70% sequence identity to the coding sequence of the nucleotides listed in SEQ ID No. 30, 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, WI 53705).

The present invention comprises a vector comprising a

DNA sequence which encodes a SCCE 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 the SCCE protein. Preferably, the vector of the present invention contains a portion of the DNA sequence shown in SEQ ID No. 30. Vectors may be used to amplify and/or express nucleic acid encoding a SCCE protein or fragment thereof.

In addition to substantially full-length proteins, the invention also includes fragments (*e.g.*, antigenic fragments) of the SCCE 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 SCCE protein can be generated by methods known to those skilled in the art, *e.g.*, by enzymatic digestion of naturally occurring or recombinant SCCE protein, by recombinant DNA techniques using an expression vector that encodes a defined fragment of SCCE, or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of SCCE (*e.g.*, binding to an antibody specific for SCCE) can be assessed by methods described herein. Purified SCCE or antigenic fragments of SCCE 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 SCCE or a fragment of SCCE 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 SCCE cDNA clones, and to distinguish them from other cDNA clones.

Further included in this invention are SCCE 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 SCCE sequence has been deleted. The fragment, or the intact SCCE 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 stratum corneum chymotrytic enzyme. 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 ^{3}H, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, 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 isothiocyalate 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 SCCE protein in a biological sample, which includes the steps of contacting the sample with the labeled antibody, *e.g.*, radioactively tagged antibody specific for stratum corneum chymotrytic enzyme, and determining whether the antibody binds to a component of the sample. Antibodies to the SCCE protein can be used in an immunoassay to detect increased levels of SCCE 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 SCCE 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 SCCE are useful in a method of detecting SCCE 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 SCCE, and detecting the SCCE 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 stratum corneum chymotrytic enzyme.

Likewise, a standard Northern blot assay can be used to ascertain the relative amounts of SCCE 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 SCCE cDNA, either containing the full-length, single stranded DNA having a sequence complementary to SEQ ID No. 30, 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 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

PCR Primers	5'→3'	SEQ ID No.
Redundant Primers:		
Serine Protease (histidine) = S1	tgggtigtiacigcigcica(ct)tg	1
Serine Protease (aspartic acid) = AS1	a(ag)ia(ag)igciatitcitticc	2
Serine Protease (serine) = AS11	a(ag)iggiccicci(cg)(ta)(ag)tcicc	3
Cysteine Protease – sense	ca(ag)ggica(ag)tg(ct)ggi(ta)(cg)itg(ct)tgg	4
Cysteine Protease - antisense	taiccicc(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
Specific Primers:		
Serine Protease (hepsin) = sense	tgtcccgatggcgagtgttt	8
Serine Protease (hepsin) = antisense	cctgttggccatagtactgc	9
Serine Protease (SCCE) = sense	agatgaatgagtacaccgtg	10
Serine Protease (SCCE) = antisense	ccagtaagtccttgtaaacc	11
Serine Protease (Comp B) = sense	aagggacacgagagctgtat	12
Serine Protease (Comp B) = antisense	aagtggtagttggaggaagc	13
Serine Protease (Protease M)= sense	ctgtgatccaccctgactat	20
Serine Protease (Protease M) = antisense	caggtggatgtatgcacact	21
Serine Protease (TADG12) = sense (Ser10-s)	gcgcactgtgtttatgagat	22
Serine Protease (TADG12) = antisense (Ser10-as)	ctctttggcttgtacttgct	23
Serine Protease (TADG13) = sense	tgagggacatcattatgcac	24
Serine Protease (TADG13) = antisense	caagttttccccataattgg	25
Serine Protease (TADG14) = sense	acagtacgcctgggagacca	26
Serine Protease (TADG14) = antisense	ctgagacggtgcaattctgg	27
Cysteine Protease (Cath-L) = sense	attggagagagaaaggctac	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. It was also shown that PCR amplification bands unique to the mucinous tumor type and the clear cell type have 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
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.

PCR products amplified from normal and carcinoma cDNAs were compared 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).

Figure 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 SCCE 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:

SCCE:

forward 5'-AGATGAATGAGTACACCGTG-3' (SEQ ID No. 10), and

reverse 5'-CCAGTAAGTCCTTGTAAACC-3' (SEQ ID No. 11);

and β-tubulin:

forward 5'- TGCATTGACAACGAGGC -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 339 bp for SCCE and 454 bp for β-tubulin. The primer sequences used in this study were designed according to

the cDNA sequences described by Hansson et al. [*J Biol. Chem.*, 269, 19420-19426 (1994)] for SCCE, 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 SCCE gene and the β-tubulin gene, 200 μmol of dNTPs, 5 μCi of α-^{32}PdCTP and 0.25 units of Taq DNA polymerase with reaction buffer (Promega) in a final volume of 25 μl. 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. It was previously established and confirmed for SCCE that co-amplification with β-tubulin under these conditions for 30 cycles remain linear for both products.

The PCR products were separated on 2% agarose gels and the radioactivity of each PCR product was determined by using a Phospho Imager (Molecular Dynamics). In the present study, expression of SCCE was calculated as the ratio (SCCE/β-tubulin) as measured by phosphoimager. The overexpression cut-off value was defined as the mean value for normal ovary +2SD. The student's t test was used for the comparison of the mean values of normal ovary and tumors.

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 (Figure 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

	Total	Stage I/II	Stage III/IV	No Stage
Serous				
Malignant	166	15	140	8
LMP	16	9	7	0
Benign	12	0	0	12
Mucinous				
Malignant	26	6	14	6
LMP	28	25	3	0
Benign	3	0	0	3
Endometrioid				
Malignant	38	17	21	0
LMP	2	2	0	0
Benign	0	0	0	0
Other*				
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 mullerian, 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.

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 μg of mRNA was loaded onto a 1% formaldehyde-agarose gel, electrophoresed and blotted onto a HyBond-N+™ nylon membrane (Amersham). ^{32}P-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 ^{32}P-labeled cDNA probe in ExpressHyb™ Hybridization Solution (CLONTECH). Control hybridization to determine relative gel loading was accomplished using the β-tubulin probe.

Normal human tissues including spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas and normal human fetal tissues; brain, lung, liver and kidney (Human Multiple Tissue Northern Blot; CLONTECH) were all examined using the same hybridization procedure.

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. Figures 3, 4 & 5 show the PCR product displays comparing normal and carcinomatous tissues using redundant primers for serine

proteases (Figure 3), for cysteine proteases (Figure 4) and for metallo-proteases (Figure 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 (Figure 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 (Figure 3, Lane 4). Compliment factor B belongs in the family of coagulation factors X (Christmas factor). As part of the intrinsic pathway, compliment factor B catalyzes the proteolytic activation of coagulation factor X in the presence of Ca^{2+} phospholipid and factor VIIIa e5; and

(c) A stratum corneum chymotryptic enzyme (SCCE) serine protease involved in desquarnation of skin cells from the human stratum corneum (Figure 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 (Figure 4, Lanes 3-5). Cloning and sequencing this PCR product identified a sequence of Cathepsin L, which is a lysomal 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

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 (Figure 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

mRNA Expression of SCCE in Ovarian Tumors

To evaluate mRNA expression of SCCE in ovarian tumors, semi-quantitative PCR was performed. A preliminary study confirmed the linearity of the PCR amplification (Shigemasa et al., *J Soc Gynecol Invest* 4, 95-102, 1997; Hall et al., *Mol Cell Biol* 3, 854-862, 1983). Figure 6 shows an example of comparative PCR using SCCE primers co-amplified with the internal control β-tubulin primers. Analysis of the data as measured using the phosphoimager and compared as ratios of expression (SCCE/β-tubulin) indicate that SCCE expression is elevated in tumor cases 19, 14, 15, 16, 43, 23, 36 and 37 compared to that of

normal ovaries.

To confirm the results of the initial quantitative PCR and to examine the size of the transcript, Northern blot hybridization was performed in representative cases of each histological type of carcinoma (Figure 7A). Northern blot hybridization with a ^{32}P-labeled SCCE probe (nucleotides 232-570) revealed 1.2 kb and 2.0 kb transcripts, as reported previously in normal skin tissue (Hansson et al., *J. Biol Chem* 269, 19420-19426, 1994). Those tumor cases which showed overexpression of SCCE by quantitative PCR also showed intense bands of SCCE transcript expression by Northern blot analysis including serous, mucinous, endometrioid and clear cell carcinoma. No transcripts were detected in normal ovarian tissue (Lane 1). Normal human tissues (spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas) and normal human fetal tissues (brain, lung, liver and kidney) examined by Northern blot analysis showed no visible SCCE transcripts (Figures 7B & 7C). Blots for normal human adult tissues and fetal tissues were subsequently probed to confirm the presence of β-tubulin transcripts.

Table 5 summarizes the results of the evaluation of SCCE expression in 10 individual normal ovarian tissues and 44 ovarian carcinomas. Overall, SCCE mRNA overexpression (overexpression = mean value for normal ovary + 2SD) was found in 8 of 12 LMP tumors (66.7%) and 25 of 32 carcinoma cases (78.1%) with p values of < 0.05 and < 0.001 respectively (Figure 8). Overexpression of SCCE transcripts was detected in all ovarian carcinoma subtypes and in both early stage and late stage tumor samples. In the five cases where positive confirmation of lymph node metastasis was identified, all five

cases showed overexpression of SCCE at a level of more than four standard deviations above the level for normal ovary. It should be noted that three of these tumors were classified as low malignant potential tumors (all serous adenomas) suggesting a possible relationship between the progression of early stage disease to the lymph when overexpression of SCCE is manifest.

TABLE 5

Patient Characteristics and Expression of SCCE Gene

Case	Histological Type[a]	Stage/Grade	LN[b]	mRNA expression[c] SCCE
1	normal ovary			n
2	normal ovary			n
3	normal ovary			n
4	normal ovary			n
5	normal ovary			n
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	2+
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	4+
19	m adenoma (LMP)	1/1	n	4+
20	m adenoma (LMP)	1/1	n	n
21	m adenoma (LMP)	1/1	NE	n
22	m adenoma (LMP)	1/1	NE	n
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	4+

29	s	carcinoma	3/3	NE	4+
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	4+
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	4+
38	s	carcinoma	3/3	n	4+
39	s	carcinoma	3/2	NE	4+
40	s	carcinoma	3/3	NE	4+
41	s	carcinoma	3/2	NE	n
42	m	carcinoma	1/2	n	n
43	m	carcinoma	2/2	NE	4+
44	m	carcinoma	2/2	n	n
45	m	carcinoma	3/1	NE	n
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+

a: s; serous, m; mucinous, e; endometrioid, c; clear cell; b: LN; lymph node metastasis, p; positive, n; negative, NE; not examined; c: n, normal range is equal to Mean ± 2SD, 2+; Mean + 2SD to + 4SD, 4+; Mean + 4SD or greater

The expression ratio (mean value ± SD) for normal ovary was determined as 0.046 ± 0.023, for LMP tumors as 0.405 ± 0.468 and for carcinoma as 0.532 ± 0.824 (Table 6). From a histological point of view, overexpression of SCCE was observed in 23 of 26 serous tumors (88.5%) including 6 of 7 LMP tumors and 17 of 19 carcinomas. However only 4 of 12 mucinous tumors (33.3%) including 2 of 5 LMP tumors and 2 of 7 carcinomas showed

overexpression of SCCE. For endometrioid and clear cell carcinoma, stratum corneum chymotrytic enzyme was found to be overexpressed in all 6 cases (Table 6).

TABLE 6

Overexpression of SCCE in Ovarian Carcinoma

	N	Overexpression of SCCE	Expression Ratio[a]
Normal	**10**	**0 (0%)**	**0.046 ± 0.023**
LMP	**12**	**8 (66.7%)**	**0.405 ± 0.468**
serous	7	6 (85.7%)	0.615 ± 0.518
mucinous	5	2 (40.0%)	0.111 ± 0.117
Carcinoma	**32**	**25 (78.1%)**	**0.532 ± 0.824**
serous	19	17 (89.5%)	0.686 ± 1.027
mucinous	7	2 (28.6%)	0.132 ± 0.265
endometrioid	3	3 (100%)	0.511 ± 0.205
clear cell	3	3 (100%)	0.515 ± 0.007

a: The ratio of expression level of SCCE to β-tubulin (mean ± SD)

EXAMPLE 14

Western Blot

Polyclonal rabbit antibodies were generated by immunization with a combination of 2 poly-lysine linked multiple Ag peptides derived from SCCE protein sequences PLQILLLSLALE (SEQ ID No. 28) and SFRHPGYSTQTH (SEQ ID No. 29). Approximately 20 ng of MDA-MBA-435S and HeLa cell lysates were separated on a 15% SDS-

PAGE gel and electroblotted to PVDF at 100 V for 40 minutes at 4°C. The proteins were fixed to the membrane by incubation in 50% MeOH for 10 minutes. The membrane was blocked overnight in TBS, pH 7.8 containing 0.2% non-fat milk. Primary antibody was added to the membrane at a dilution of 1:100 in 0.2% milk/TBS and incubated for 2 hours at room temperature. The blot was washed and incubated with a 1:3000 dilution of alkaline-phosphatase conjugated goat anti-rabbit IgG (BioRad) for one hour at room temperature. The blot was washed and incubated with a chemiluminescent substrate before a 10 second exposure to X-ray film for visualization.

Two cell lines HeLa and MDA-MB-435S previously shown to express mRNA transcripts were examined by Western blot to confirm the presence of SCCE protein. Figure 9 indicates that polyclonal antibodies developed to peptides (12 mers bound to polylysine) derived from the amino and carboxy termini of SCCE bind a protein of approximately 30 kDa in cytosolic extracts of HeLa and MDA-MB-435S cells. The ovarian tumor cell line CAOV3 was also examined for SCCE expression and a protein product could not be detected (data not shown). This molecular size protein agrees with the anticipated and known parameters for the SCCE protein. It should be noted that only a single band was detected by Western blot analysis of cystosolic protein. It might be anticipated that the SCCE protein prior to secretion would be present in the inactivated parent form i.e. the seven amino terminal peptide removed on activation would still be present on the enzyme. In this pre-active form of the enzyme it would be anticipated that the apparent molecular weight on Western blot would be about 30 kDa.

EXAMPLE 15

Immunohistochemistry

Immunohistochemical localization of SCCE antigen was examined using normal ovaries, mucinous LMP tumor and adenocarcinomas (including serous adenocarcinomas, mucinous adenocarcinoma and clear cell carcinomas) in the same series of the samples for mRNA isolation. Formalin fixed and paraffin embedded sections, 4 μm thick, were cut and mounted on aminopropyltriethoxysilane treated slides. Slides were routinely deparaffinized with xylene and rehydrated with a series of ethanol washes. Nonenzymatic antigen retrieval was performed by processing using microwave heat treatment in 0.01 M sodium citrate buffer (pH 6.0). Immunohistochemical staining was performed manually using the avidin-biotin peroxidase complex technique (Vectastain Elite ABC kit, Vector Laboratories). Anti-SCCE rabbit polyclonal antibody was generated by immunization with a combination of 2 poly-lysine linked multiple Ag peptide derived from the SCCE protein-sequences.

This indirect immunoperoxidase staining procedure was performed at room temperature. Endogenous peroxidase and nonspecific background staining were blocked by incubating slides with methanol with 0.3% H_2O_2 for 30 minutes. After washing with phosphate-buffered saline (PBS) for 10 minutes, sections were incubated with biotinylated anti-rabbit IgG for 30 minutes. After washing with PBS for 10 minutes, slides were incubated with ABC reagent for 30 minutes. The final products were visualized by using AEC substrate system (DAKO Corporation) and sections were counterstained with Mayer hematoxylin for 20 seconds before

mounting. Positive controls and negative controls were used for each section. Negative controls were performed by using normal rabbit serum instead of the primary antibody. All experiments were duplicated. The stained slides were examined microscopically by 3 observers. More than 10% of positive tumor cells was the criterion for a 1+ positive staining and more than 50% of positive tumor cells was the criterion for a 2+ positive staining.

To further confirm the presence of the SCCE protein in ovarian tumor cells as opposed to its elaboration by supporting stromal or blood vessel cells, both normal ovarian epithelia and ovarian tumor tissue were examined by immunohistochemistry using the polyclonal antiserum (described above). All 14 ovarian tumors showed positive staining of SCCE, whereas normal ovarian surface epithelium showed very weak expression of SCCE antigen (Figure 10A). 8 of 10 serous adenocarcinomas, 1 of 1 mucinous adenocarcinoma, and 2 of 2 clear cell carcinomas showed 2+ positive staining (more than 50% of positive tumor cells) of SCCE (Table 7). Figures 10C and 10E show that stratum corneum chymotrytic enzyme staining is localized to the cytoplasm and the cell membrane of ovarian tumor cells. The negative control of each case was also performed, wherein the result showed no nonspecific staining of stratum corneum chymotrytic enzyme (Figures 10B, 10D and 10F) and staining of normal ovarian epithelial cells which showed little SCCE expression (Figure 10A).

TABLE 7

Immunohistochemical Expression of SCCE Protein in Normal Ovary and Ovarian Tumor

Lab No.	Histology	SCCE
	normal ovary	weak +
	normal ovary	weak +
	normal ovary	weak +
	normal ovary	weak +
	normal ovary	weak +
	normal ovary	weak +
1036	mucinous LMP	+
475	serous carcinoma	+
465	serous carcinoma	++
464	serous carcinoma	++
1039	serous carcinoma	++
960	serous carcinoma	++
962	serous carcinoma	++
1551	serous carcinoma	++
1813	serous carcinoma	++
1817	serous carcinoma	+
1819	serous carcinoma	++
1244	mucinous carcinoma	++
947	clear cell carcinoma	++
948	clear cell carcinoma	++

EXAMPLE 16

Summary of Known Proteases Detected Herein

Most of the above-listed proteases were identified from the sense-His/antisense-Ser primer pair, yielding a 500 bp PCR product (Figure 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. Hepsin 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).

Figure 11A shows that hepsin was expressed in ovarian carcinomas of different histologic types, but not in normal ovary. Figure 11B 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. Figure 11C shows that hepsin overexpression is not observed in normal adult tissue. Slight expression above the background level is observed in the adult prostate. 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.

Hepsin expression was investigated in normal (N), mucinous (M) and serous (S) low malignant potential (LMP) tumors and carcinomas (CA). Figure 12A shows quantitative PCR of hepsin and internal control β-tubulin. Figure 12B shows a bar graph of expression of hepsin 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 Figures 12A & 12B). 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 (Figures 7A, 7B and 7C). 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 (Figure 7A). 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 (Figure 6). Figure 8 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 cloned by Anisowicz, *et al.*, and shown to be overexpressed in carcinomas. A evaluation indicates that this

enzyme is overexpressed in ovarian carcinoma (Figure 13).

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 17

Summary of Previously Unknown Proteases Detected Herein

TADG-12

TADG-12 was identified from the primer pairs, sense-His/antisense-Asp (see Figure 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 Figure 14). 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 (Figure 15A) 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 (Figure 15B). 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 TADG-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 metallo-protease 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.

MMP-7 or PUMP-1 is differentially expressed in fetal lung and kidney tissues. Figure 16A compares PUMP-1 expression in normal ovary and carcinoma subtypes using Northern blot analysis. Notably, PUMP-1 is expressed in ovarian carcinoma tissues, and again, the presence of a transcript in normal tissue was not detected. Figure 16B shows the expression of PUMP-1 in human fetal tissue, while no transcript could be detected in either fetal brain or fetal liver. Figure 16C shows that PUMP-1 overexpression is not observed in normal adult tissue. 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 (Figures 17A & 17B). PUMP-1, however, is so far the protease most frequently found overexpressed in mucinous tumors (See Table 8 below).

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 (Figure 18). However, its presence or absence in combination with other proteases of the present invention permits identification of specific tumor types and treatment choices.

Conclusion

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 M, TADG12, TADG14 and the metallo-protease PUMP-1 (see Figure 19 and Table 8). Northern blot analysis of normal and ovarian carcinoma tissues 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	Hepsin	SCCE	Pump-1	Protease 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)

Discussion

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 gene's 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.

Specifically, the present invention utilizes primers to the conserved catalytic triad domain of the serine protease family (viz. His--Asp--Ser). Using such a strategy to display serine protease transcripts found in abundance in carcinoma tissues, with little or no expression in normal ovary, SCCE gene was detected.

The overall expectation of the search was to identify cell surface or secreted products which may promote either tumor growth or metastasis. Confirmation of the presence of SCCE (a secreted serine protease) in ovarian tumors was indicated initially by subcloning and sequencing PCR products derived from amplification of tumor cDNA using redundant primes to the histidine (sense) and the serine (antisense) conserved domains of the serine protease catalytic sequences. Characterization of the SCCE protease (Egelrud, T. *J Invest Dermatol* 101, 200-204, 1993) indicated that the cohesion between individual corneocytes in the stratum comeurn, the primary substrate for cellular desquamation or shedding of skin cells may be degraded by SCCE. Proteolysis of these intercellular matrices is one of the major events preceding desquamation. SCCE has only been identified in the stratum comeurn (Egelrud, T. *J Invest Dermatol* 101, 200-204, 1993; Hansson, et al., *J Biol Chem* 269, 19420-19426, 1994) and immunohistochemical studies confirmed its unique tissue specific expression by the epithelial cells of the stratum comeurn (SondeII, et al., *J Histochem Cytochem* 42, 459-465, 1994). It was therefore surprising to discover that this highly conserved expression of SCCE to skin is obviated when transformation and carcinogenesis of ovarian epithelial cells occurs. The clearly distinctive pattern of expression in both low malignant potential tumors and overt carcinomas of the

ovary over normal ovarian tissue suggests that the SCCE protease may also play a role in shedding or desquamation of ovarian tumor cells. This association is especially well preserved in serous adenocarcinomas where disease progression is characterized by early foci of peritoneal metastasis and which may be the result of an early overexpression of enzymes such as SCCE and consequent tumor cell shedding. Because SCCE and other proteases (e.g. hepsin) are overexpressed in ovarian tumors (again with particularly high overexpression in serous tumors) it seems likely that a concert of lytic activity at the cell surface may be involved in malignant potential. Several aspects of the tumorigenic process can be dissected and identified as component parts of such a surface protease concert viz 1) initial expansion of newly transformed cells into the surrounding matrix of supporting tissue of the primary organ; 2) desquamation or shedding of tumor cells into the surrounding environment; 3) invasion of basement membrane of the target organ of metastasis; and 4) activation of mitogenic and angiogenic factors to support the newly established metastatic colony.

While it is not yet clear which proteases are the primary agents in each of these malignant progression steps, the data here indicate the potential for the involvement of SCCE in the shedding or desquamation phase of this progression. Certain other factors remain to be resolved even with regard to SCCE involvement in tumor cell shedding which include activation of SCCE by proteolysis or cleaving of the aminoterminal peptide of the pro-protease. Furthermore, an antileukoprotease which specifically inhibits SCCE activity has been recently identified (Wiedow, O. (1995) *Isolierung und*

Charakterisierung von Serinprotease Inhibitoren der menschlichen Epidermis, Köster, Berlin). The presence of such an inhibitor might effectively inhibit shedding or desquamation of tumor cells as it has been shown to inhibit the detachment of corneocytes of keratinized skin tissue.

While there remains an intricate interaction between surface protease expression/activation and/or inhibition, it appears likely that a concert of enzymes which contribute to tumor .growth and spread provide a mechanism for such a progression. SCCE expression on ovarian tumor cell surfaces can provide one mechanism by which tumor cells may be shed early in the tumor progression process of serous carcinomas.

The unique presence of this protease to keratinized stratum comeum and the present data showing lack of transcript presence in all normal adult and fetal tissues examined support the potential of this secreted extracellular enzyme as a useful marker for ovarian carcinoma. The fact that inhibition of such an activity prevents normal desquamation of skin cells also points to the potential of SCCE as a target for inhibition or down regulation in therapeutic intervention in the spread or metastasis of ovarian carcinoma.

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.

WHAT IS CLAIMED IS:

1. A method of diagnosing a cancer in an individual, comprising the steps of:

(a) obtaining a biological sample from said individual; and

(b) detecting stratum corneum chymotrypsin enzyme in said sample, wherein the presence of stratum corneum chymotrytic enzyme in said sample is indicative of the presence of a cancer in said individual, wherein the absence of stratum corneum chymotrytic enzyme in said sample is indicative of the absence of a cancer in said individual.

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

3. The method of claim 1, wherein said detection of said stratum corneum chymotrytic enzyme is by means selected from the group consisting of Northern blot, Western blot, PCR, dot blot, ELIZA sandwich assay, radioimmunoassay, DNA array chips and flow cytometry.

4. The method of claim 1, wherein said cancer is selected from the group consisting of ovarian, breast, lung, colon, prostate and others in which stratum corneum chymotrytic enzyme is overexpressed.

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

(a) isolating mRNA from said sample; and

(b) detecting stratum corneum chymotrytic enzyme mRNA in said sample, wherein the presence of said stratum corneum chymotrytic enzyme mRNA in said sample is indicative of the presence of malignant hyperplasia, wherein the absence of said stratum corneum chymotrytic enzyme mRNA in said sample is indicative of the absence of malignant hyperplasia.

6. The method of claim 5, further comprising the step of:

comparing said stratum corneum chymotrytic enzyme mRNA to reference information, wherein said comparison provides a diagnosis of said malignant hyperplasia.

7. The method of claim 5, further comprising the step of:

comparing said stratum corneum chymotrytic enzyme mRNA to reference information, wherein said comparison determines a treatment of said malignant hyperplasia.

8. The method of claim 5, wherein said detection of said stratum corneum chymotrytic enzyme mRNA is by PCR amplification.

9. The method of claim 8, wherein said PCR amplification uses primers selected from the group consisting of SEQ ID No. 10 and SEQ ID No. 11.

10. The method of claim 5, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

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

(a) isolating protein from said sample; and

(b) detecting stratum corneum chymotrytic enzyme protein in said sample, wherein the presence of said stratum corneum chymotrytic enzyme protein in said sample is indicative of the presence of malignant hyperplasia, wherein the absence of said stratum corneum chymotrytic enzyme protein in said sample is indicative of the absence of malignant hyperplasia.

12. The method of claim 11, further comprising the step of:

comparing said stratum corneum chymotrytic enzyme protein to reference information, wherein said comparison provides a diagnosis of said malignant hyperplasia.

13. The method of claim 11, further comprising the step of:

comparing said stratum corneum chymotrytic enzyme protein to reference information, wherein said comparison determines a treatment of said malignant hyperplasia.

14. The method of claim 11, wherein said detection is by immunoaffinity to an antibody, wherein said antibody is specific for stratum corneum chymotrytic enzyme.

15. The method of claim 11, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

16. A method of inhibiting expression of endogenous stratum corneum chymotrytic enzyme in a cell, comprising the step of:

introducing a vector into a cell, wherein said vector comprises a stratum corneum chymotrytic enzyme gene in opposite orientation operably linked to elements necessary for expression, wherein expression of said vector in said cell produces stratum corneum chymotrytic enzyme antisense mRNA, wherein said stratum corneum chymotrytic enzyme antisense mRNA hybridizes to endogenous stratum corneum chymotrytic enzyme mRNA, thereby inhibiting expression of endogenous stratum corneum chymotrytic enzyme in said cell.

17. A method of inhibiting stratum corneum chymotrytic enzyme protein in a cell, comprising the step of:

introducing an antibody into a cell, wherein said antibody is specific for a stratum corneum chymotrytic enzyme protein or a fragment thereof, wherein binding of said antibody to said stratum corneum chymotrytic enzyme protein inhibits said stratum corneum chymotrytic enzyme protein.

18. A method of targeted therapy to an individual, comprising the step of:

administering a compound to an individual, wherein said compound has a targeting moiety and a therapeutic moiety, wherein said targeting moiety is specific for stratum corneum chymotrytic enzyme.

19. The method of claim 18, wherein said targeting moiety is selected from the group consisting of an antibody specific for stratum corneum chymotrytic enzyme and a ligand or ligand binding domain that binds stratum corneum chymotrytic enzyme.

20. The method of claim 18, wherein said therapeutic moiety is selected from the group consisting of a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant and a cytotoxic agent.

21. The method of claim 18, wherein said individual suffers from a disease selected from the group consisting of ovarian cancer, lung cancer, prostate cancer, colon cancer and other cancers in which stratum corneum chymotrytic enzyme is overexpressed.

22. A method of vaccinating an individual against stratum corneum chymotrytic enzyme, comprising the step of:

inoculating an individual with a stratum corneum chymotrytic enzyme protein or fragment thereof, wherein said stratum corneum chymotrytic enzyme protein or fragment thereof lack stratum corneum chymotrytic enzyme protease activity, wherein said inoculation with said stratum corneum chymotrytic enzyme protein or fragment thereof elicits an immune response in said individual, thereby vaccinating said individual against stratum corneum chymotrytic enzyme.

23. The method of claim 22, wherein said individual has a cancer, is suspected of having a cancer or is at risk of getting a cancer.

24. The method of claim 22, wherein said stratum corneum chymotrytic enzyme fragment is selected from the group consisting of a 9-residue fragment up to a 20-residue fragment.

25. The method of claim 24, wherein said 9-residue fragment is selected from the group consisting of SEQ ID Nos. 31, 32, 33, 34, 35, 36, 80, 86 and 99.

26. A method of producing immune-activated cells directed toward stratum corneum chymotrytic enzyme, comprising the steps of:

exposing dendritic cells to a stratum corneum chymotrytic enzyme protein or fragment thereof, wherein said stratum corneum chymotrytic enzyme protein or fragment thereof lacks stratum corneum chymotrytic enzyme protease activity, wherein said exposure to said stratum corneum chymotrytic enzyme protein or fragment thereof activates said dendritic cells, thereby producing immune-activated cells directed toward stratum corneum chymotrytic enzyme.

27. The method of claim 26, wherein said immune-activated cells are selected from the group consisting of B-cells, T-cells and dendrites.

28. The method of claim 26, wherein said stratum corneum chymotrytic enzyme fragment is selected from the group consisting of a 9-residue fragment up to a 20-residue fragment.

29. The method of claim 28, wherein said 9-residue fragment is selected from the group consisting of SEQ ID Nos. 31, 32, 33, 34, 35, 36, 80, 86 and 99.

30. The method of claim 26, wherein said dendritic cells are isolated from an individual prior to said exposure, wherein said activated dendritic cells are reintroduced into said individual subsequent to said exposure.

31. The method of claim 30, wherein said individual has a cancer, is suspected of having a cancer or is at risk of getting a cancer.

32. An immunogenic composition, comprising an immunogenic fragment of a stratum corneum chymotrytic enzyme protein and an appropriate adjuvant.

33. The immunogenic composition of claim 32, wherein said stratum corneum chymotrytic enzyme fragment is selected from the group consisting of a 9-residue fragment up to a 20-residue fragment.

34. The immunogenic composition of claim 33, wherein said 9-residue fragment is selected from the group consisting of SEQ ID Nos. 31, 32, 33, 34, 35, 36, 80, 86 and 99.

35. An oligonucleotide having a sequence complementary to SEQ ID No. 30.

36. A composition comprising the oligonucleotide of claim 35 and a physiologically acceptable carrier.

37. A method of treating a neoplastic state in an individual in need of such treatment, comprising the step of:

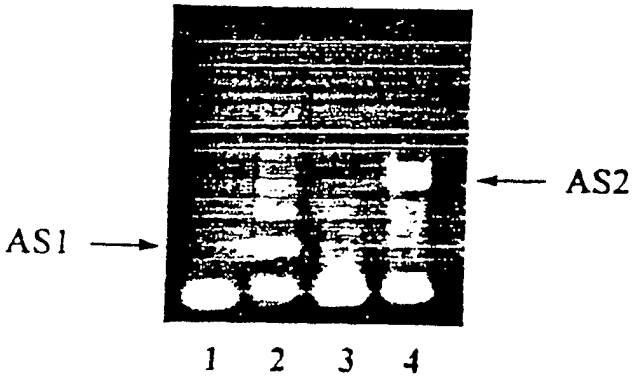
administering to said individual an effective dose of the oligonucleotide of claim 35.

38. The method of claim 37, wherein said neoplastic state is selected from the group consisting of ovarian cancer, breast cancer, lung cancer, colon cancer, prostate cancer and other cancers in which stratum corneum chymotrytic enzyme is overexpressed.

39. A method of screening for compounds that inhibit stratum corneum chymotrytic enzyme activity, comprising the steps of:

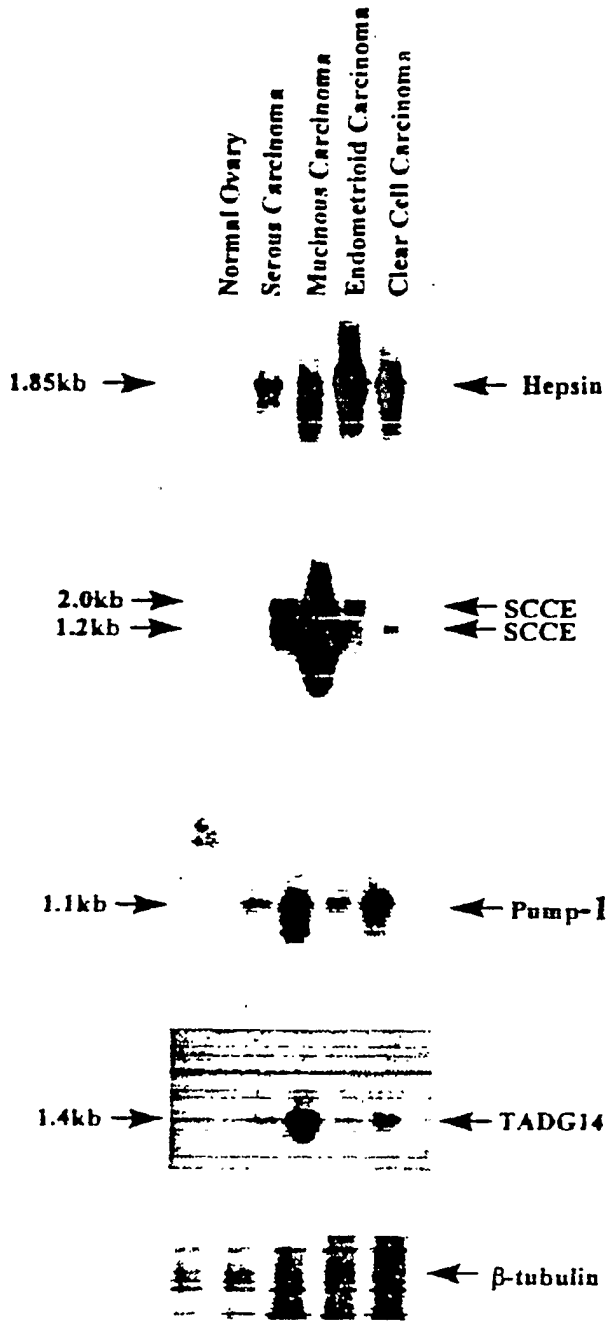
contacting a sample with a compound, wherein said sample comprises stratum corneum chymotrytic enzyme protein; and

assaying for stratum corneum chymotrytic enzyme protease activity, wherein a decrease in said stratum corneum chymotrytic enzyme protease activity in the presence of said compound relative to stratum corneum chymotrytic enzyme protease activity in the absence of said compound indicatives the compound inhibits stratum corneum chymotrytic enzyme activity.



1) Normal Ovary 2) Tumor
3) Normal Ovary 4) Tumor

Fig. 1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SCCE
1.1kb
Pump-1
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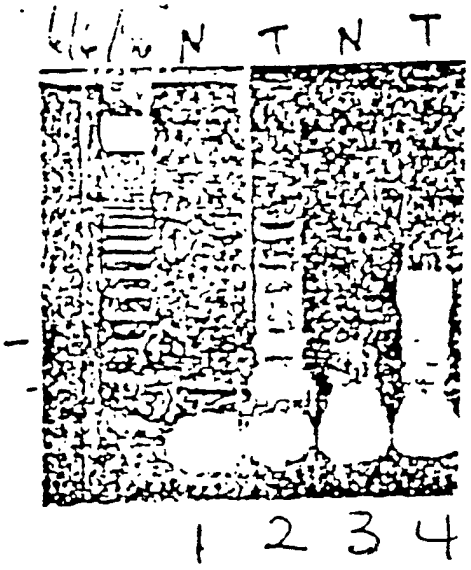
TADG14
β-tubulin

Fig. 2



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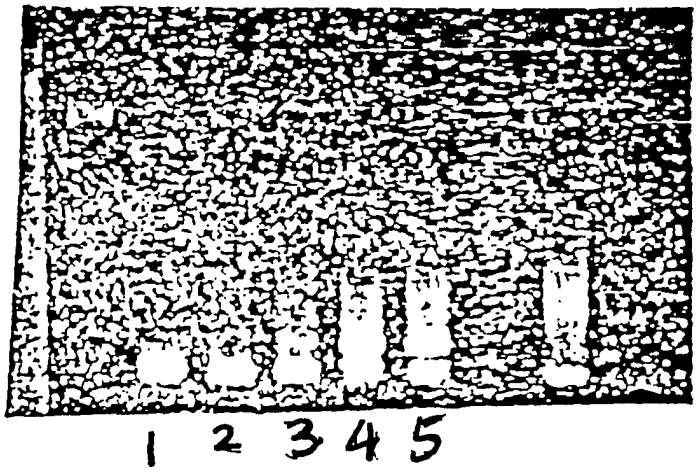


Fig. 4

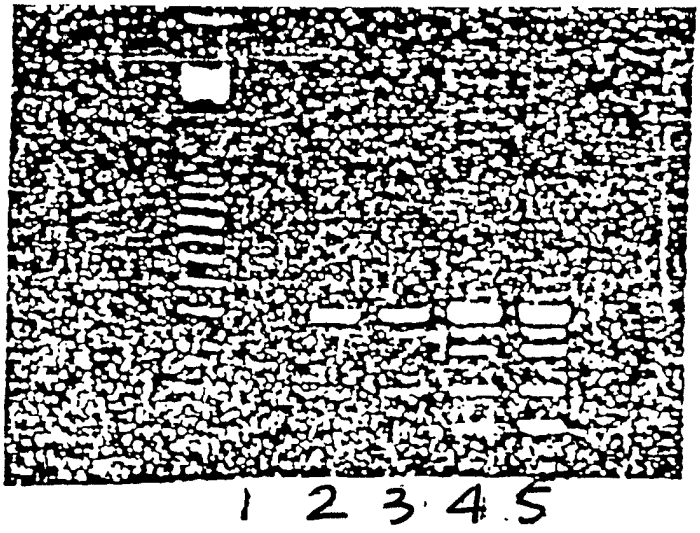
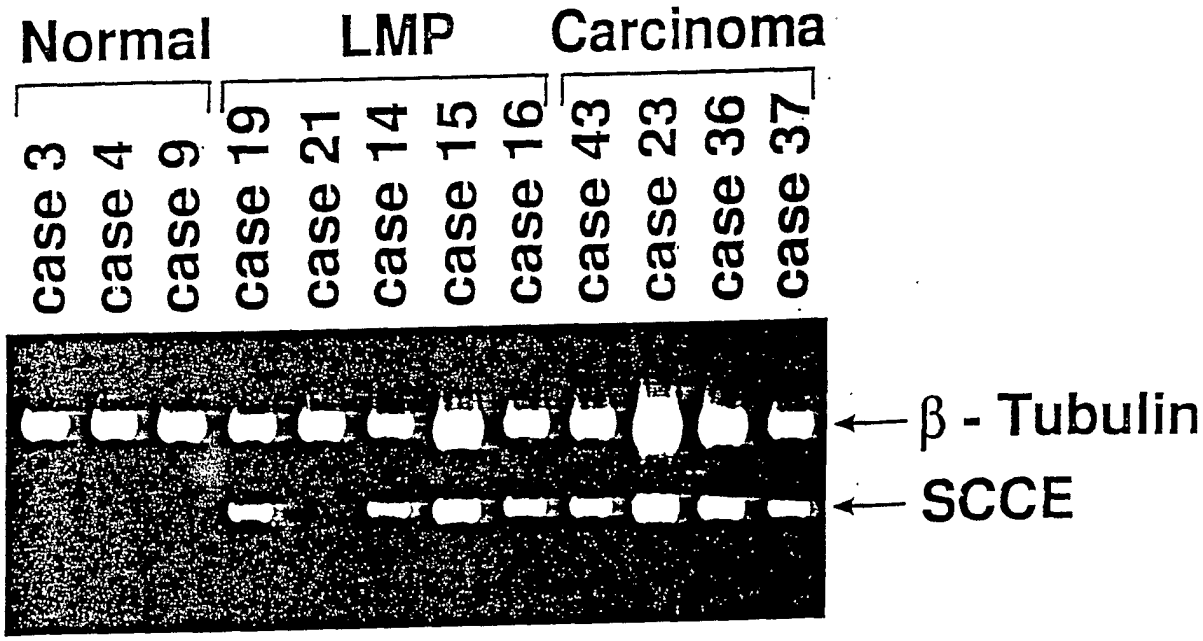


Fig. 5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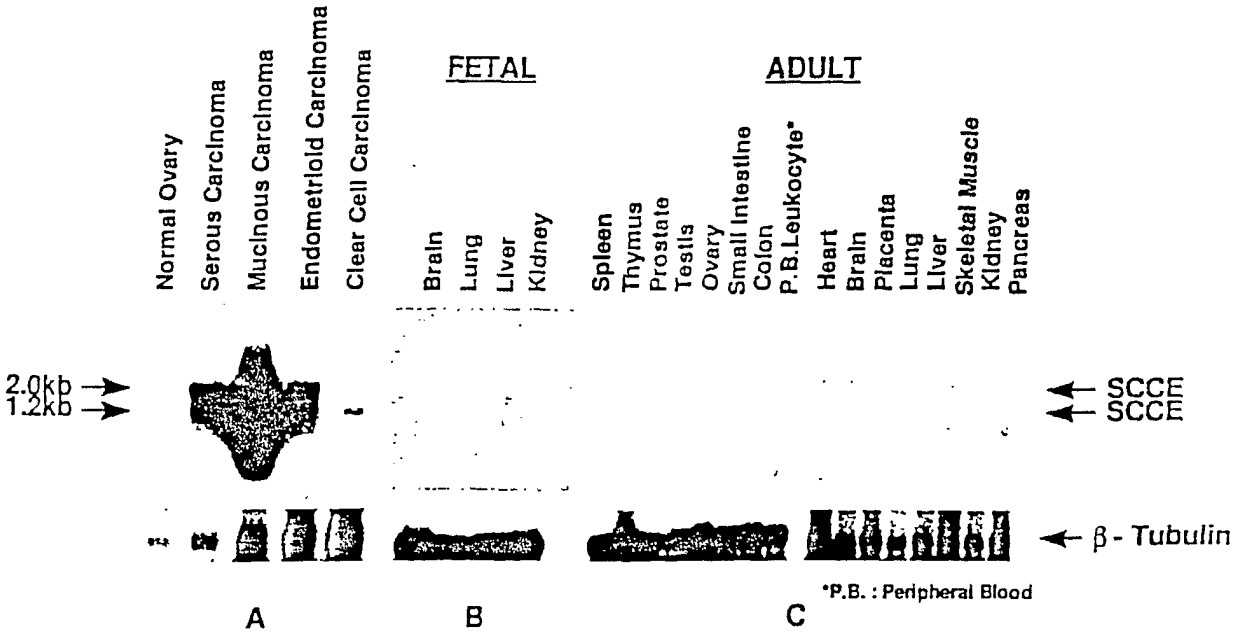
Carcinoma
case 3
case 4
case 9
case 19
case 21
case 14
case 15
case 16
case 43
case 23
case 36
case 37
β - Tubulin
SCCE

Fig. 6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Clear Cell Carcinoma
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Lung
Liver
Kidney
ADULT
Spleen
Thymus
Prostate
Testis
Ovary
Small Intestine
Colon
P.B.Leukocyte*
Heart
Brain
Placenta
Lung
Liver
Skeletal Muscle
Kidney
Pancreas
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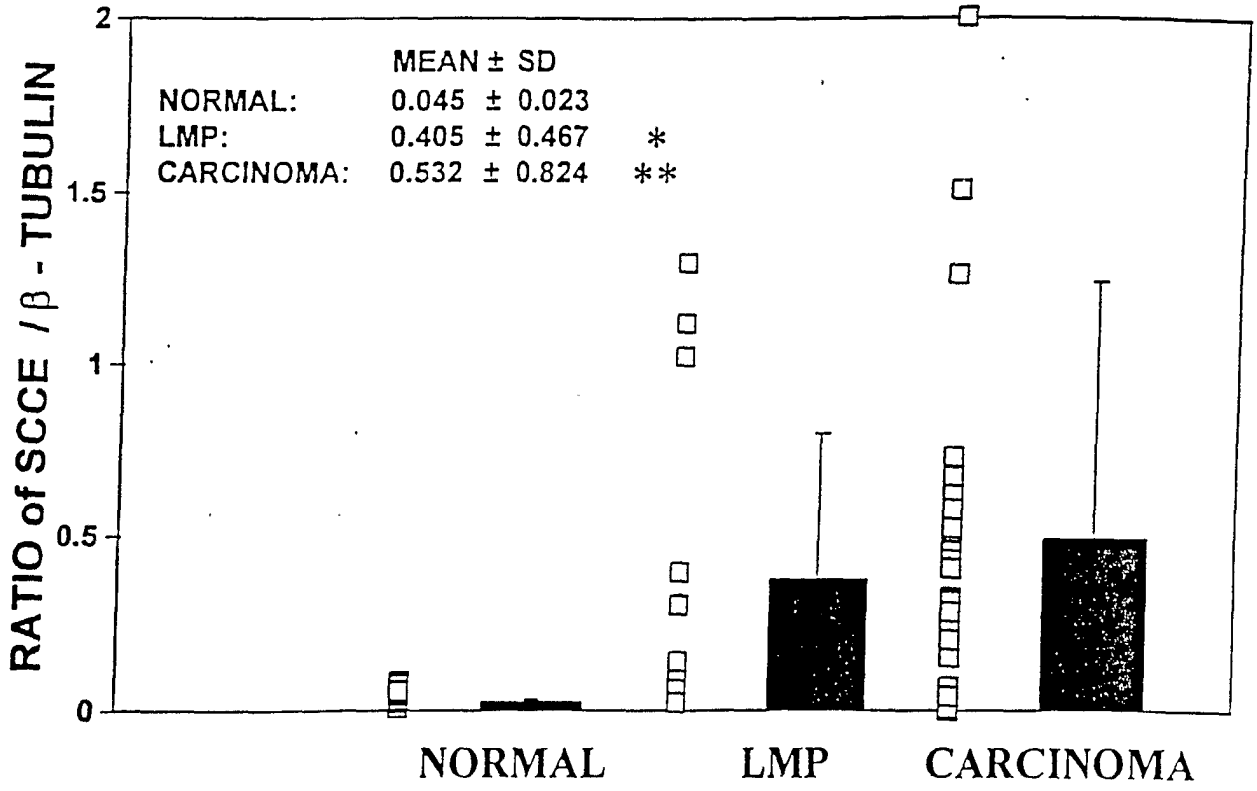
1.2kb
SCCE
SCCE
β - Tubulin
*P.B. : Peripheral Blood
A
B
C

Fig. 7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2
1.5
1
0.5
0
MEAN ± SD
NORMAL: 0.045 ± 0.023
LMP: 0.405 ± 0.467 *
CARCINOMA: 0.532 ± 0.824 **
NORMAL
LMP
CARCINOMA

Fig. 8

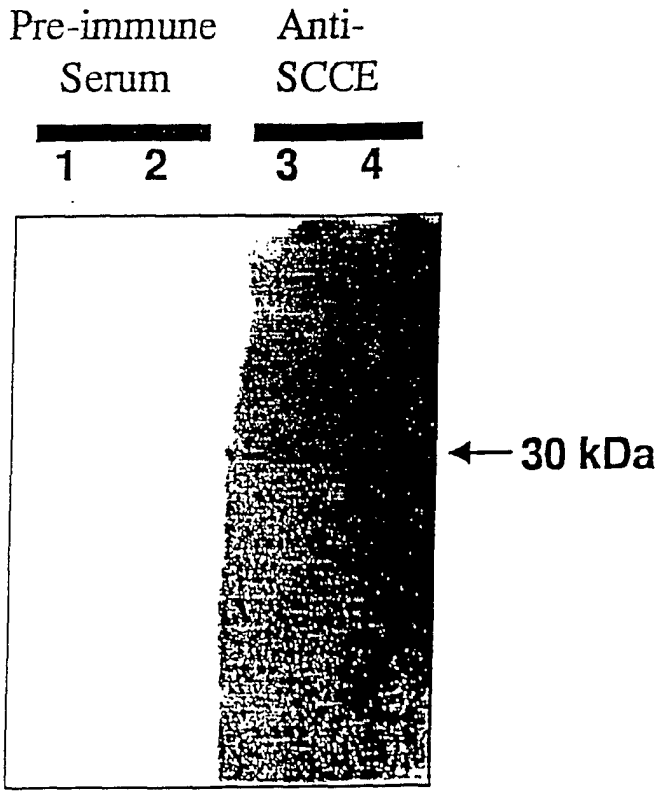


Fig. 9

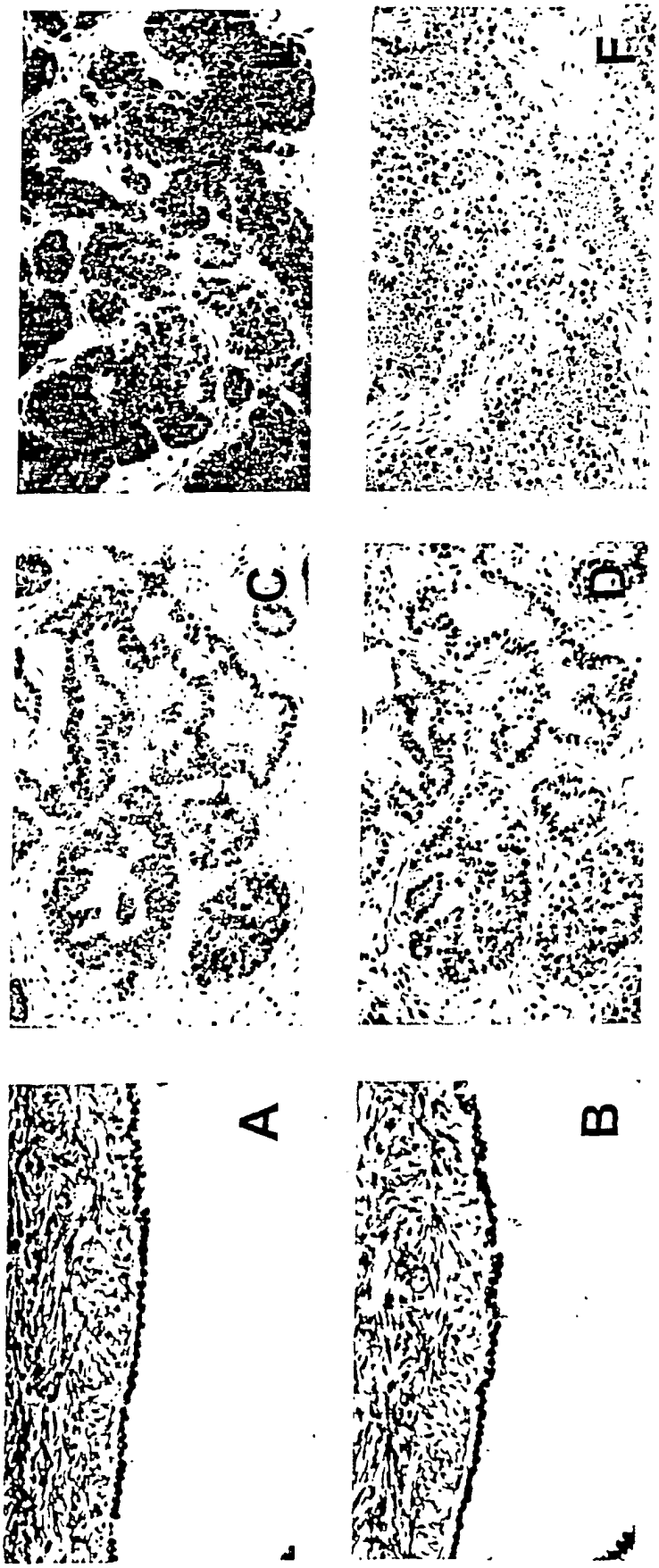
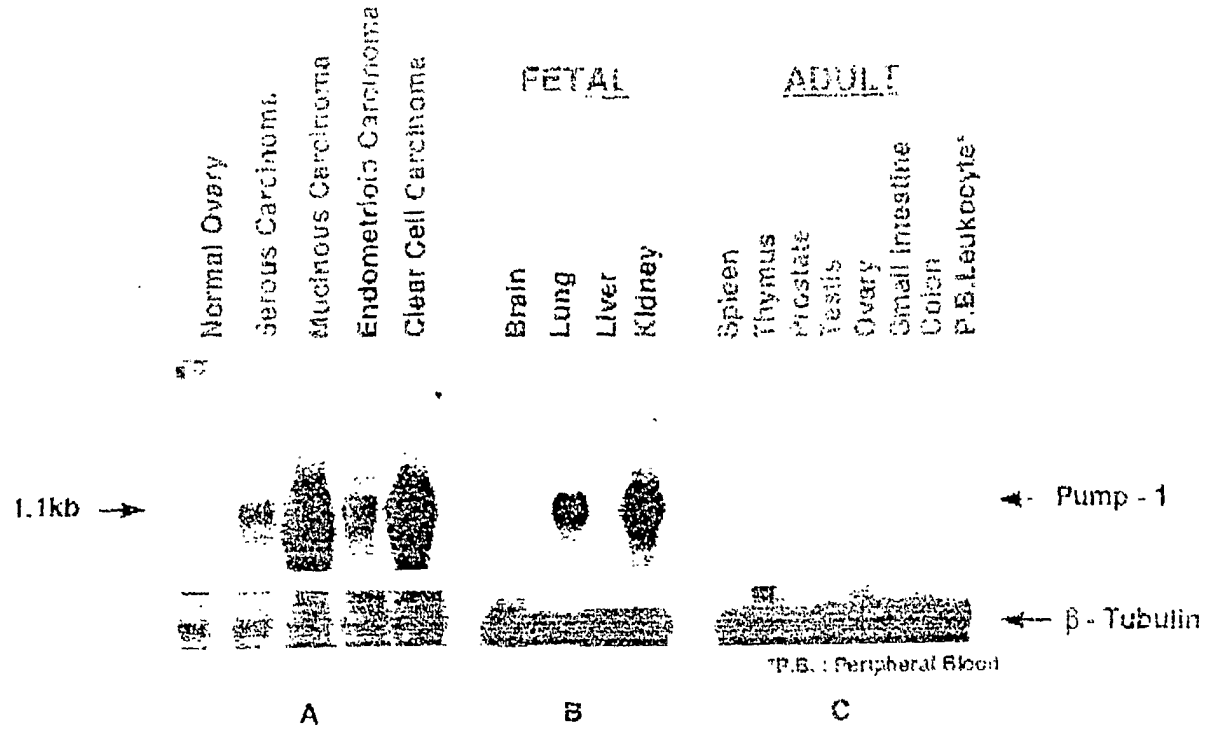


Fig. 10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ADULT
Spleen
Thymus
Prostate
Testis
Ovary
Small Intestine
Colon
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1.1kb
Pump - 1
β - Tubulin
*P.B. : Peripheral Blood
A
B
C

Fig. 11

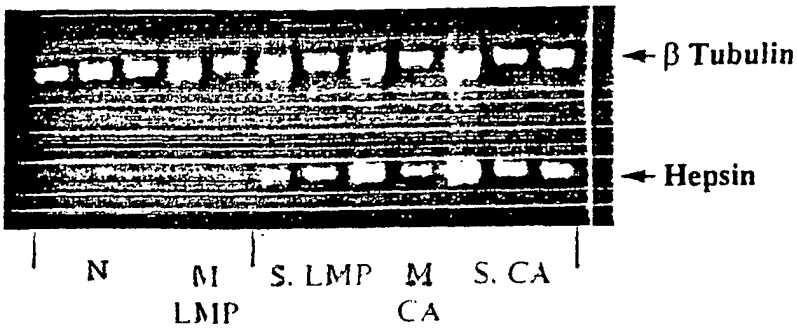

Fig. 12A

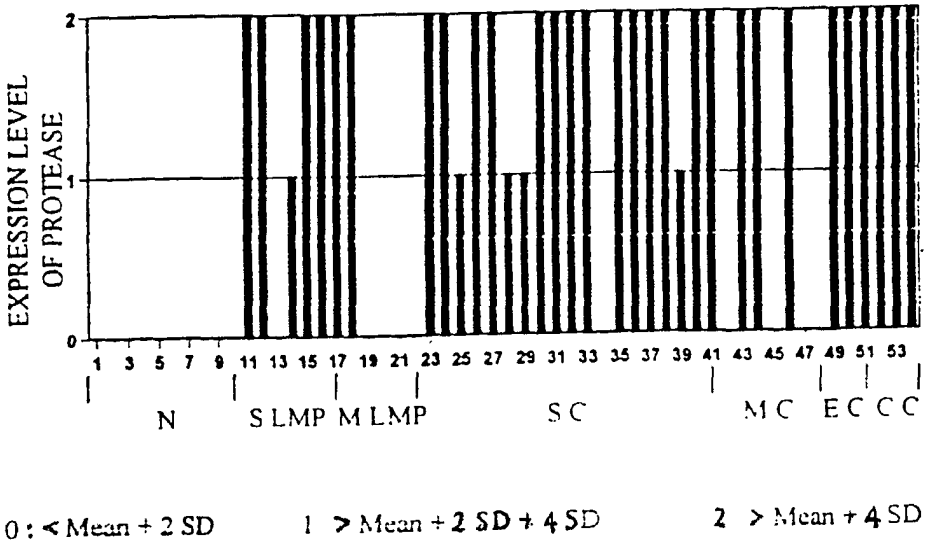

Fig. 12B

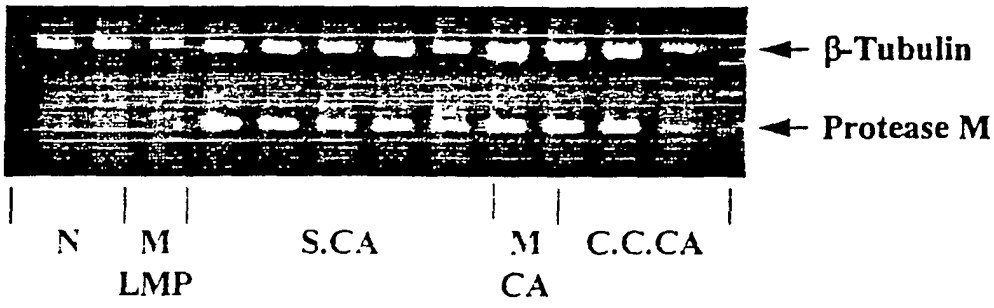

Fig. 13

1. .15

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16 .30

SWTIQVGLVSLLDNP

31 .45

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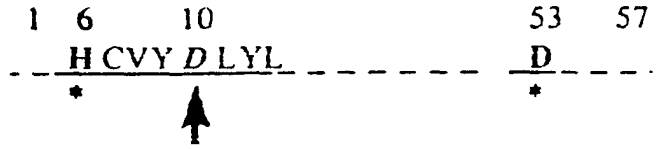
46 57

KPKRLGNDIALL

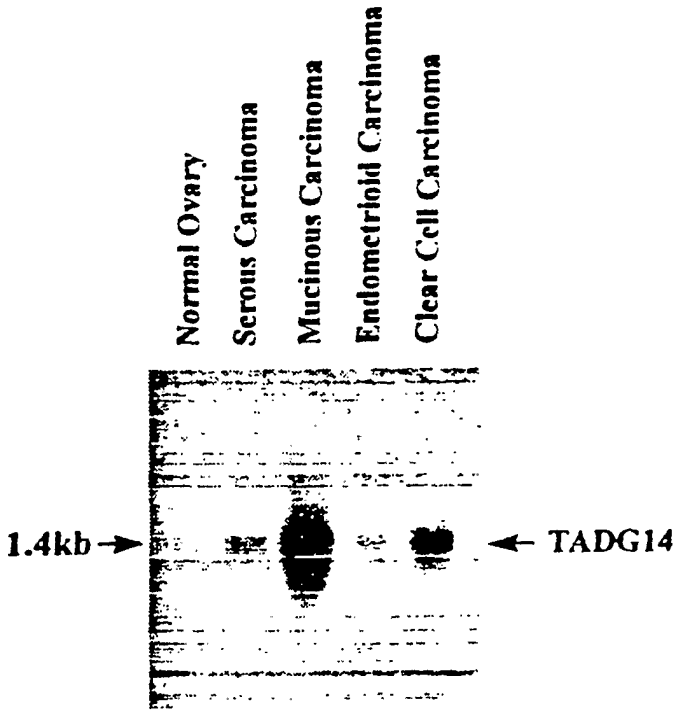
↓ indicates the site of insert in TADG12

H & D are the conserved regions of Serine protease.



site of 133 bp insert in TADG12

Fig. 14



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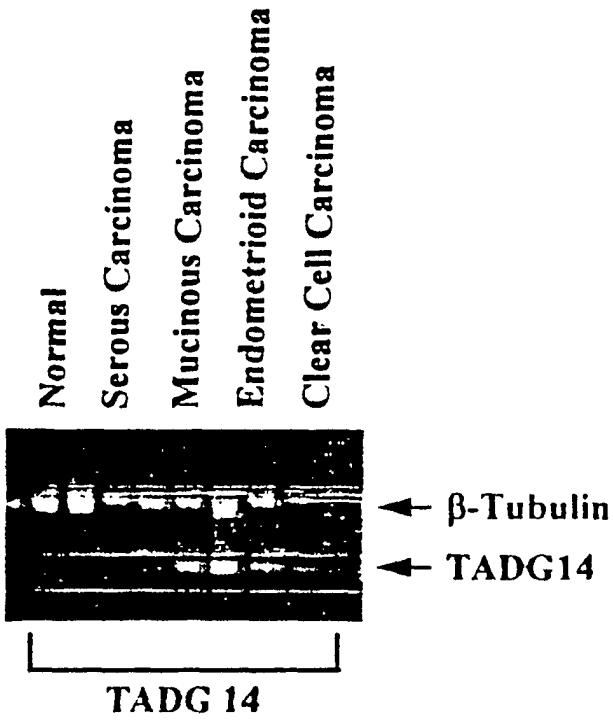

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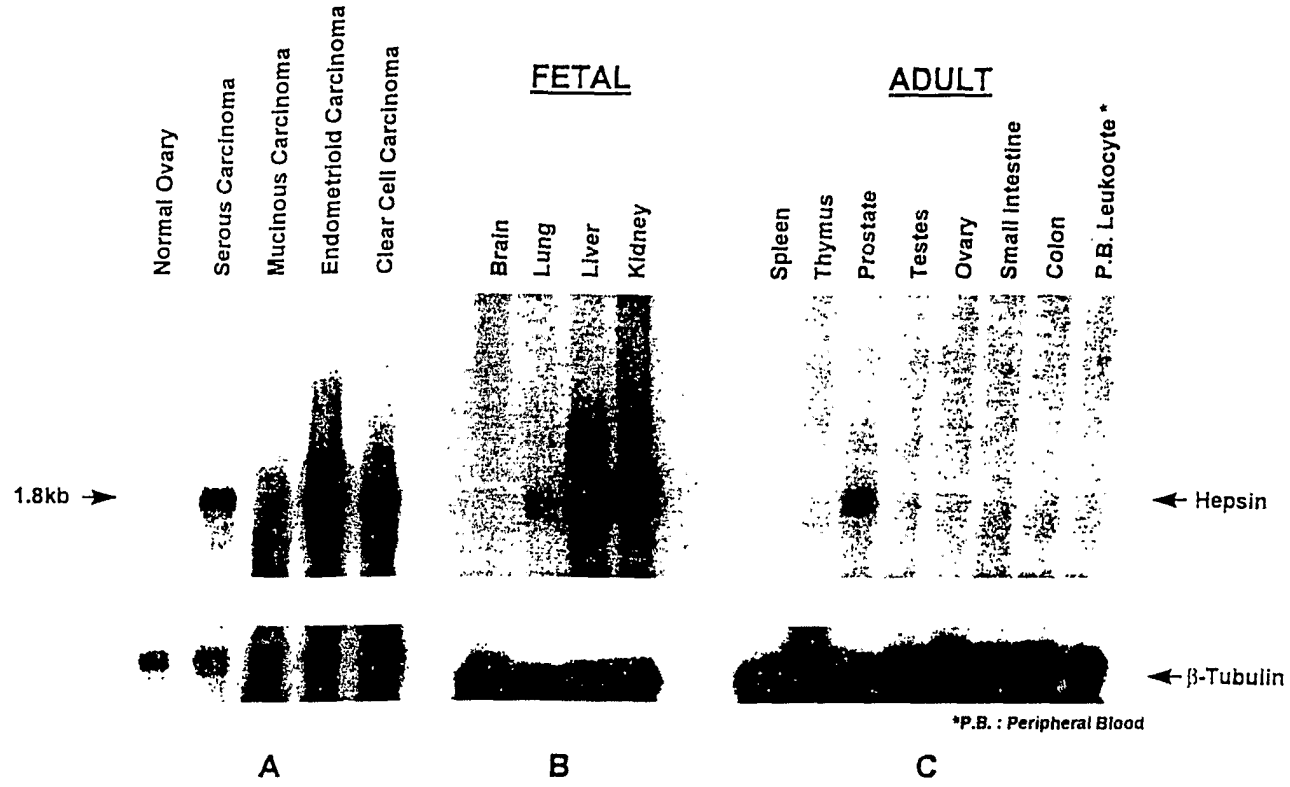
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Thymus
Prostate
Testes
Ovary
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1.8kb
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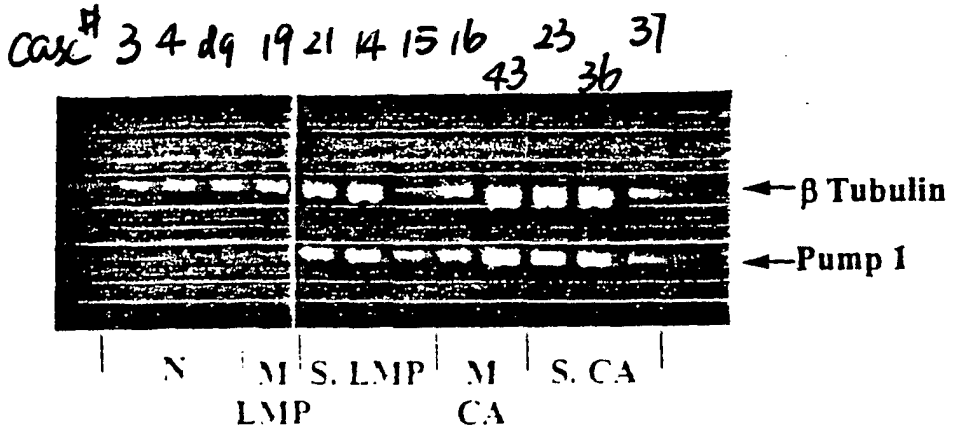
*P.B. : Peripheral Blood
A
B
C

Fig. 16



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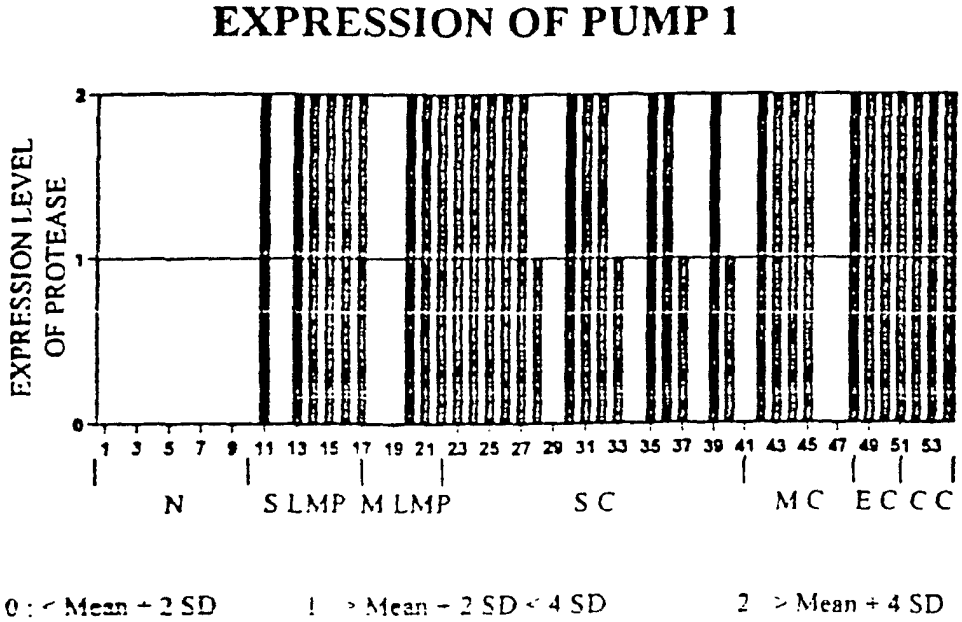

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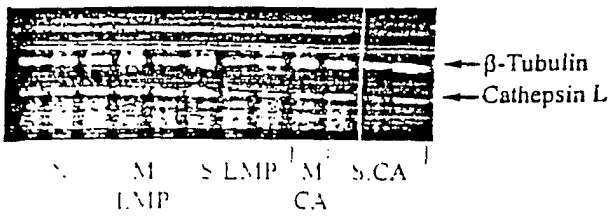
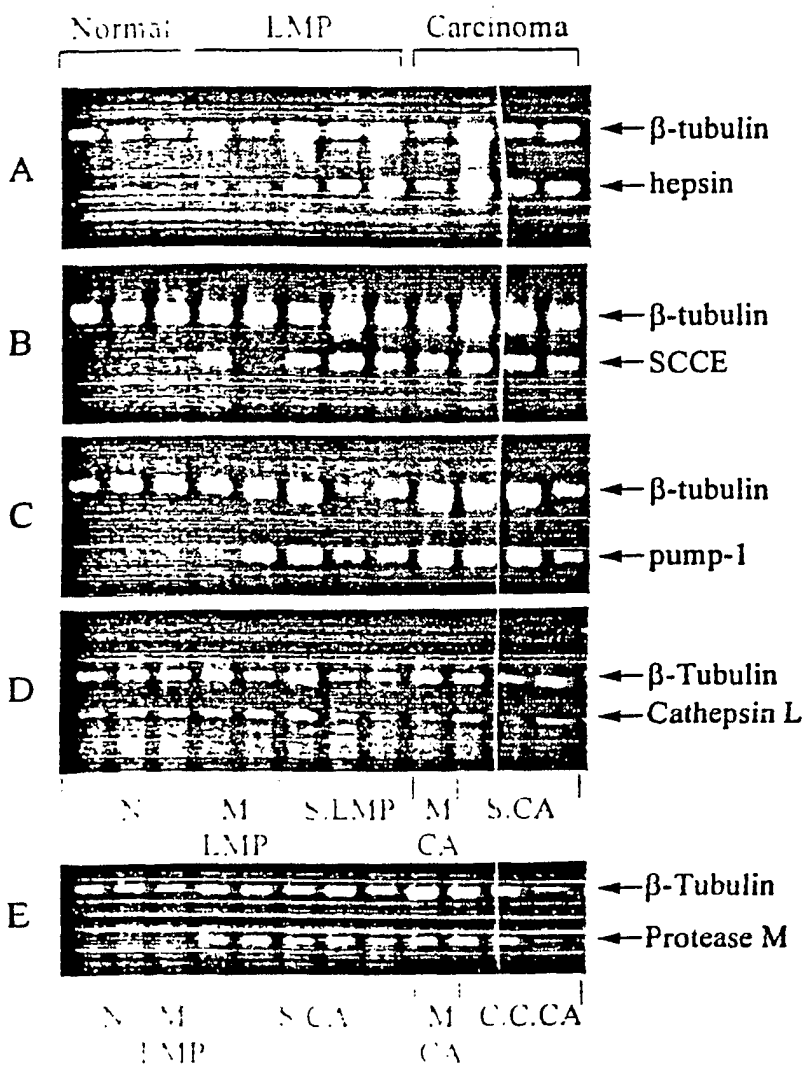


Fig. 18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Cathepsin L
N
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Fig. 19

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tgagggacat cattatgcac 20

<210> 25
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> antisense oligonucleotide primer specific for TADG-13

<400> 25
caagttttcc ccataattgg 20

<210> 26
<211> 20
<212> DNA
<213> Artificial sequence

<220>

<223> sense oligonucleotide primer specific for TADG-14

<400> 26
acagtacgcc tgggagacca 20

<210> 27
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> antisense oligonucleotide primer specific for TADG-14

<400> 27
ctgagacggt gcaattctgg 20

<210> 28
<211> 12
<212> PRT
<213> Artificial sequence

<220>
<223> a poly-lysine linked multiple Ag peptide derived from SCCE protein sequences

<400> 28
Pro Leu Gln Ile Leu Leu Leu Ser Leu Ala Leu Glu
5 10

<210> 29
<211> 12
<212> PRT
<213> Artificial sequence

<220>
<223> a poly-lysine linked multiple Ag peptide derived from SCCE protein sequences

<400> 29
Ser Phe Arg His Pro Gly Tyr Ser Thr Gln Thr His
5 10

<210> 30
<211> 969
<212> DNA
<213> *Homo sapiens*

<220>
<223> full length cDNA of SCCE

<400> 30
ttgagggttt tgtgtttctt tatttgtttt ggttttaggt ctttaccaat 50
ttgattggtt tatcaacagg gcatgaggtt taaatatatc tttgaggaaa 100
ggtaaagtca aatttgactt cataggtcat cggcgtcctc actcctgtgc 150

attttctgtt ggaagcacac agttaattaa ctcagtgtgg cgttagcgat 200
gctttttcat ggtgtcattt atccacttgg tgaacttgca cacttgagtg 250
tagactcctg ggtcattggg ttggccgcaa gggaaagttc cccaggacac 300
cagaccttgc agggtacctc tgcacaccaa cggtcccct gagtcaccat 350
tgcaggcgtt tttcttggag tcggggatgc cagcgcacag catggaattt 400
tccagtaagt ccttgtaaac cttcgtgcag tcctgggggg agatgagctt 450
gacatccacg cacatgaggt cagagggaaa ggtcacatct gggctcgtgg 500
tagtgcccca gccggagaca gtacaggtgg ttccaggggg ttcgcagcgg 550
gagggcagcc tgactttctt caccatggat gacagcctgg cctggctatt 600
gagcttcacg agcatgaggt cattaacatg ggtctgtgtg gagtagccgg 650
ggtggcggaa tgacttcgag gccttgatcc tctgagctct cctgtcgccc 700
agcgtatcac tgcccaggtg cacggtgtac tcattcatct tgcagtgggc 750
ggcagtgagc acccagcgct cattgaccag gacgcctccg cagtggagct 800
gattgccact gagcagggcc acctgccatg ggtgggagcc tcttgcacat 850
ggggcgccat caataatctt gtcaccctgg gcttcttctc ctgcagtttc 900
caaggctaag gatagcagta ggatctgcag gggcaggaga agggatcttg 950
ccatggagcc cggaaatcc 969

<210> 31
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 72-80 of the SCCE protein

<400> 31
Lys Met Asn Glu Tyr Thr Val His Leu
5

<210> 32
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 123-131 of the SCCE protein

<400> 32
Arg Leu Ser Ser Met Val Lys Lys Val
5

<210> 33
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 5-13 of the SCCE protein

<400> 33
Leu Leu Leu Pro Leu Gln Ile Leu Leu
5

<210> 34
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 58-66 of the SCCE protein

<400> 34
Val Leu Val Asn Glu Arg Trp Val Leu
5

<210> 35
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 6-14 of the SCCE protein

<400> 35
Leu Leu Pro Leu Gln Ile Leu Leu Leu
5

<210> 36
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 4-12 of the SCCE protein

<400> 36
Ser Leu Leu Leu Pro Leu Gln Ile Leu
5

<210> 37
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 52-60 of the SCCE protein

<400> 37
Gln Leu His Cys Gly Gly Val Leu Val
5

<210> 38
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>

<223> Residues 12-20 of the SCCE protein

<400> 38
Leu Leu Leu Ser Leu Ala Leu Glu Thr
5

<210> 39
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 163-171 of the SCCE protein

<400> 39
Leu Met Cys Val Asp Val Lys Leu Ile
5

<210> 40
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 57-65 of the SCCE protein

<400> 40
Gly Val Leu Val Asn Glu Arg Trp Val
5

<210> 41
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 237-245 of the SCCE protein

<400> 41
Gln Val Cys Lys Phe Thr Lys Trp Ile
5

<210> 42
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 169-177 of the SCCE protein

<400> 42
Lys Leu Ile Ser Pro Gln Asp Cys Thr
5

<210> 43
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 10-18 of the SCCE protein

<400> 43
Gln Ile Leu Leu Leu Ser Leu Ala Leu
5

<210> 44
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 29-37 of the SCCE protein

<400> 44
Lys Ile Ile Asp Gly Ala Pro Cys Ala
5

<210> 45
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 215-223 of the SCCE protein

<400> 45
Leu Gln Gly Leu Val Ser Trp Gly Thr
5

<210> 46
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 13-21 of the SCCE protein

<400> 46
Leu Leu Ser Leu Ala Leu Glu Thr Ala
5

<210> 47
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>

<223> Residues 114-122 of the SCCE protein

<400> 47
Met Leu Val Lys Leu Asn Ser Gln Ala
5

<210> 48
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 47-55 of the SCCE protein

<400> 48
Leu Leu Ser Gly Asn Gln Leu His Cys
5

<210> 49
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 65-73 of the SCCE protein

<400> 49
Val Leu Thr Ala Ala His Cys Lys Met
5

<210> 50
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 59-67 of the SCCE protein

<400> 50
Leu Val Asn Glu Arg Trp Val Leu Thr
5

<210> 51
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 51-59 of the SCCE protein

<400> 51
Asn Gln Leu His Cys Gly Gly Val Leu
5

<210> 52
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 77-85 of the SCCE protein

<400> 52
Thr Val His Leu Gly Ser Asp Thr Leu
5

<210> 53
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 45-53 of the SCCE protein

<400> 53
Val Ala Leu Leu Ser Gly Asn Gln Leu
5

<210> 54
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 162-170 of the SCCE protein

<400> 54
Asp Leu Met Cys Val Asp Val Lys Leu
5

<210> 55
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 218-226 of the SCCE protein

<400> 55
Leu Val Ser Trp Gly Thr Phe Pro Cys
5

<210> 56
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 145-153 of the SCCE protein

<400> 56
Thr Val Ser Gly Trp Gly Thr Thr Thr
5

<210> 57
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 136-144 of the SCCE protein

<400> 57
Arg Cys Glu Pro Pro Gly Thr Thr Cys
5

<210> 58
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 81-89 of the SCCE protein

<400> 58
Gly Ser Asp Thr Leu Gly Asp Arg Arg
5

<210> 59
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 30-38 of the SCCE protein

<400> 59
Ile Ile Asp Gly Ala Pro Cys Ala Arg
5

<210> 60
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 183-191 of the SCCE protein

<400> 60
Leu Leu Glu Asn Ser Met Leu Cys Ala
5

<210> 61
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 21-29 of the SCCE protein

<400> 61
Ala Gly Glu Glu Ala Gln Gly Asp Lys
5

<210> 62
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 235-243 of the SCCE protein

<400> 62
Tyr Thr Gln Val Cys Lys Phe Thr Lys
5

<210> 63
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 170-178 of the SCCE protein

<400> 63
Leu Ile Ser Pro Gln Asp Cys Thr Lys
5

<210> 64
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 245-253 of the SCCE protein

<400> 64
Ile Asn Asp Thr Met Lys Lys His Arg
5

<210> 65
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 157-165 of the SCCE protein

<400> 65
Val Thr Phe Pro Ser Asp Leu Met Cys
5

<210> 66
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 109-117 of the SCCE protein

<400> 66
His Val Asn Asp Leu Met Leu Val Lys
5

<210> 67
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 17-25 of the SCCE protein

<400> 67
Ala Leu Glu Thr Ala Gly Glu Glu Ala
5

<210> 68
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 151-159 of the SCCE protein

<400> 68
Thr Thr Thr Ser Pro Asp Val Thr Phe
5

<210> 69
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 68-76 of the SCCE protein

<400> 69
Ala Ala His Cys Lys Met Asn Glu Tyr
5

<210> 70
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 173-181 of the SCCE protein

<400> 70
Pro Gln Asp Cys Thr Lys Val Tyr Lys
5

<210> 71
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 204-212 of the SCCE protein

<400> 71
Asp Ser Gly Gly Pro Leu Val Cys Arg
5

<210> 72
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 39-47 of the SCCE protein

<400> 72
Gly Ser His Pro Trp Gln Val Ala Leu
5

<210> 73
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 222-230 of the SCCE protein

<400> 73
Gly Thr Phe Pro Cys Gly Gln Pro Asn
5

<210> 74
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 165-173 of the SCCE protein

<400> 74
Cys Val Asp Val Lys Leu Ile Ser Pro
5

<210> 75
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 110-118 of the SCCE protein

<400> 75
Val Asn Asp Leu Met Leu Val Lys Leu
5

<210> 76
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 179-187 of the SCCE protein

<400> 76
Val Tyr Lys Asp Leu Leu Glu Asn Ser
5

<210> 77
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 105-113 of the SCCE protein

<400> 77
Ser Thr Gln Thr His Val Asn Asp Leu
5

<210> 78
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 234-242 of the SCCE protein

<400> 78

Val Tyr Thr Gln Val Cys Lys Phe Thr
5

<210> 79
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 125-133 of the SCCE protein

<400> 79
Ser Ser Met Val Lys Lys Val Arg Leu
5

<210> 80
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 207-215 of the SCCE protein

<400> 80
Gly Pro Leu Val Cys Arg Gly Thr Leu
5

<210> 81
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 51-59 of the SCCE protein

<400> 81
Asn Gln Leu His Cys Gly Gly Val Leu
5

<210> 82
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 175-183 of the SCCE protein

<400> 82
Asp Cys Thr Lys Val Tyr Lys Asp Leu
5

<210> 83
<211> 9

<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 103-111 of the SCCE protein

<400> 83
Gly Tyr Ser Thr Gln Thr His Val Asn
5

<210> 84
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 201-209 of the SCCE protein

<400> 84
Cys Asn Gly Asp Ser Gly Gly Pro Leu
5

<210> 85
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 210-218 of the SCCE protein

<400> 85
Val Cys Arg Gly Thr Leu Gln Gly Leu
5

<210> 86
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 1-9 of the SCCE protein

<400> 86
Met Ala Arg Ser Leu Leu Leu Pro Leu
5

<210> 87
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 125-133 of the SCCE protein

<400> 87
Ser Ser Met Val Lys Lys Val Arg Leu
5

<210> 88
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 156-164 of the SCCE protein

<400> 88
Asp Val Thr Phe Pro Ser Asp Leu Met
5

<210> 89
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 72-80 of the SCCE protein

<400> 89
Lys Met Asn Glu Tyr Thr Val His Leu
5

<210> 90
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 107-115 of the SCCE protein

<400> 90
Gln Thr His Val Asn Asp Leu Met Leu
5

<210> 91
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 176-184 of the SCCE protein

<400> 91
Cys Thr Lys Val Tyr Lys Asp Leu Leu
5

<210> 92
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 138-146 of the SCCE protein

<400> 92
phe Pro Pro Gly Thr Thr Cys Thr Val
5

<210> 93
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 70-78 of the SCCE protein

<400> 93
His Val Lys Met Asn Glu Tyr Thr Val
5

<210> 94
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 175-183 of the SCCE protein

<400> 94
Asp Cys Thr Lys Val Tyr Lys Asp Leu
5

<210> 95
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 119-127 of the SCCE protein

<400> 95
Asn Ser Gln Ala Arg Leu Ser Ser Met
5

<210> 96
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>

<223> Residues 241-249 of the SCCE protein

<400> 96
Phe Thr Lys Trp Ile Asn Asp Thr Met
5

<210> 97
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 90-98 of the SCCE protein

<400> 97
Ala Gln Arg Ile Lys Ala Ser Lys Ser
5

<210> 98
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 238-246 of the SCCE protein

<400> 98
Val Cys Lys Phe Thr Lys Trp Ile Asn
5

<210> 99
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 91-99 of the SCCE protein

<400> 99
Gln Arg Ile Lys Ala Ser Lys Ser Phe
5

<210> 100
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 62-70 of the SCCE protein

<400> 100
Glu Arg Trp Val Leu Thr Ala Ala His
5

<210> 101
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 211-219 of the SCCE protein

<400> 101
Cys Arg Gly Thr Leu Gln Gly Leu Val
5

<210> 102
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 135-143 of the SCCE protein

<400> 102
Ser Arg Cys Glu Pro Pro Gly Thr Thr
5

<210> 103
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 37-45 of the SCCE protein

<400> 103
Ala Arg Gly Ser His Pro Trp Gln Val
5

<210> 104
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 227-235 of the SCCE protein

<400> 104
Gly Gln Pro Asn Asp Pro Gly Val Tyr
5

<210> 105
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 236-244 of the SCCE protein

<400> 105
Thr Gln Val Cys Lys Phe Thr Lys Trp
5

<210> 106
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 88-96 of the SCCE protein

<400> 106
Arg Arg Ala Gln Arg Ile Lys Ala Ser
5

<210> 107
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 87-95 of the SCCE protein

<400> 107
Asp Arg Arg Ala Gln Arg Ile Lys Ala
5

<210> 108
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 233-241 of the SCCE protein

<400> 108
Gly Val Tyr Thr Gln Val Cys Lys Phe
5

<210> 109
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 72-80 of the SCCE protein

<400> 109
Lys Met Asn Glu Tyr Thr Val His Leu
5

<210> 110
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 122-130 of the SCCE protein

<400> 110
Ala Arg Leu Ser Ser Met Val Lys Lys
5

<210> 111
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 120-128 of the SCCE protein

<400> 111
Ser Gln Ala Arg Leu Ser Ser Met Val
5

<210> 112
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 9-17 of the SCCE protein

<400> 112
Leu Gln Ile Leu Leu Leu Ser Leu Ala
5

<210> 113
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 215-223 of the SCCE protein

<400> 113
Leu Gln Gly Leu Val Ser Trp Gly Thr
5

<210> 114
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 131-139 of the SCCE protein

<400> 114
Val Arg Leu Pro Ser Arg Cys Glu Pro
5

<210> 115
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 106-114 of the SCCE protein

<400> 115
Thr Gln Thr His Val Asn Asp Leu Met
5

<210> 116
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 2-10 of the SCCE protein

<400> 116
Ala Arg Ser Leu Leu Leu Pro Leu Gln
5

<210> 117
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 99-107 of the SCCE protein

<400> 117
Phe Arg His Pro Gly Tyr Ser Thr Gln
5

<210> 118
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 137-145 of the SCCE protein

<400> 118
Cys Glu Pro Pro Gly Thr Thr Cys Thr
5

<210> 119
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 61-69 of the SCCE protein

<400> 119
Asn Glu Arg Trp Val Leu Thr Ala Ala
5

<210> 120
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 172-180 of the SCCE protein

<400> 120
Ser Pro Gln Asp Cys Thr Lys Val Tyr
5

<210> 121
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 23-31 of the SCCE protein

<400> 121
Glu Glu Ala Gln Gly Asp Lys Ile Ile
5

<210> 122
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 74-82 of the SCCE protein

<400> 122
Asn Glu Tyr Thr Val His Leu Gly Ser
5

<210> 123
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 22-30 of the SCCE protein

<400> 123
Gly Glu Glu Ala Gln Gly Asp Lys Ile
5

<210> 124
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 216-224 of the SCCE protein

<400> 124
Gln Gly Leu Val Ser Trp Gly Thr Phe
5

<210> 125
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 32-40 of the SCCE protein

<400> 125
Asp Gly Ala Pro Cys Ala Arg Gly Ser
5

<210> 126
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
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<210> 127
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<213> *Homo sapiens*

<220>
<223> Residues 227-235 of the SCCE protein

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<210> 128
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<213> *Homo sapiens*

<220>
<223> Residues 111-119 of the SCCE protein

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<210> 129
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<213> *Homo sapiens*

<220>
<223> Residues 191-199 of the SCCE protein

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Ala Gly Ile Pro Asp Ser Lys Lys Asn
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<210> 130
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<220>
<223> Residues 91-99 of the SCCE protein

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<210> 131
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<213> *Homo sapiens*

<220>
<223> Residues 236-244 of the SCCE protein

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Thr Gln Val Cys Lys Phe Thr Lys Trp
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<210> 132
<211> 9
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<213> *Homo sapiens*

<220>
<223> Residues 82-90 of the SCCE protein

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<210> 133
<211> 9
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<213> *Homo sapiens*

<220>
<223> Residues 151-159 of the SCCE protein

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Thr Thr Thr Ser Pro Asp Val Thr Phe
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<210> 134
<211> 9
<212> PRT
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<220>
<223> Residues 181-189 of the SCCE protein

<400> 134
Lys Asp Leu Leu Glu Asn Ser Met Leu
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<210> 135
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 213-221 of the SCCE protein

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Gly Thr Leu Gln Gly Leu Val Ser Trp
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<210> 136
<211> 9
<212> PRT
<213> *Homo sapiens*

<220>
<223> Residues 141-149 of the SCCE protein

<400> 136
Gly Thr Thr Cys Thr Val Ser Gly Trp
5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/03977

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 7.4, 91.2, 212, 226; 536/23.2, 23.5, 24.31, 24.33; 530/350; 514/8, 12, 44; 424/ 138.1, 185.1, 277.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/41656 A (T.J. O'BRIEN) 24 September 1998, pages 4, 6, 20-21.	1-39
X	US 5,834,290 A (EGELRUD et al) 10 November 1998, columns 14-15, 20, 23-24.	16-21, 26-30, 32-36, 39

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
12 MARCH 2001	18 APR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CARLA MYERS Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/03977

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C12Q 1/68; C12P 19/34; C07H 21/04; C07K 14/435; C12N 9/64; A61K 38/17, 38/43, 48/00; G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 7.1, 7.4, 91.2, 212, 226; 536/23.2, 23.5, 24.31, 24.33; 530/350; 514/8, 12, 44; 424/ 138.1, 185.1, 277.1

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST: US, EP, JP, WO Patents; DIALOG: Medline, CA, Biosis, Embase, Scisearch; GenBank, EMBL, Gene-seq search terms: SCCE, stratum corneum chymotryptic enzyme, HSCCE, PRSS6, KLK7, cancer, tumor, hyperplasia, malignant, ovarian; SEQ ID NO: 10, 11, 30-36, 80, 86, 99

专利名称(译)	用于卵巢癌早期诊断的组合物和方法		
公开(公告)号	EP1254267A4	公开(公告)日	2005-01-26
申请号	EP2001907085	申请日	2001-02-07
申请(专利权)人(译)	阿肯色大学的董事会		
当前申请(专利权)人(译)	阿肯色大学的董事会		
[标]发明人	OBRIEN TIMOTHY J		
发明人	O'BRIEN, TIMOTHY, J.		
IPC分类号	A61K38/00 A61K39/00 A61K48/00 C07K14/435 C12N9/64 C12Q1/37 C12Q1/68 G01N33/573 G01N33/574 C12P19/34 C07H21/04 A61K38/17 A61K38/43 G01N33/53		
CPC分类号	C12N9/6424 A61K38/00 A61K39/00 A61K48/00 A61K2039/5154 A61K2039/53 C07K14/435 C12N9/6472 C12Q1/37 C12Q1/686 C12Q1/6886 C12Q2600/136 C12Q2600/158 G01N33/573 G01N33/574 G01N33/57449 G01N33/57492 G01N2333/96486 G01N2333/976		
代理机构(译)	WILKINSON，STEPHEN JOHN		
优先权	09/502600 2000-02-11 US		
其他公开文献	EP1254267A1		
外部链接	Espacenet		

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。