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(72) Inventeurs/Inventors:
O'BRIEN, TIMOTHY J., US;
UNDERWOOD, LOWELL J., US

(73) Propriétaire/Owner:

(54) Titre : SERINE PROTEASE TRANSMEMBRANAIRE SUREXPRIMEE DANS LE CARCINOME OVARIEN ET SES APPLICATIONS
(54) Title: TRANSMEMBRANE SERINE PROTEASE OVEREXPRESSED IN OVARIAN CARCINOMA AND USES THEREOF

(57) Abrégé/Abstract:

The present invention provides a TADG-12 protein and a DNA fragment encoding such protein. Also provided is a vector/host cell capable of expressing the DNA. The present invention further provided various methods of early detection of associated ovarian and other malignancies, and of interactive therapies for cancer treatment by utilizing the DNA and/or protein disclosed herein.

(73) **Propriétaires(suite)/Owners(continued):**

THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ARKANSAS SYSTEM, US

(74) **Agent:** BORDEN LADNER GERVAIS LLP

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5 **TRANSMEMBRANE SERINE PROTEASE OVEREXPRESSED IN**
 OVARIAN CARCINOMA AND USES THEREOF

BACKGROUND OF THE INVENTION

10 Field of the Invention

 The present invention relates generally to the fields of cellular biology and diagnosis of neoplastic disease. More specifically, the present invention relates to a transmembrane serine protease termed Tumor Associated Differentially-Expressed
15 Gene-12 (TADG-12), which is overexpressed in ovarian carcinoma.

Description of the Related Art

 Tumor cells rely on the expression of a concert of proteases to be released from their primary sites and move to
20 distant sites to inflict lethality. This metastatic nature is the result of an aberrant expression pattern of proteases by tumor cells and also by stromal cells surrounding the tumors [1-3]. For most tumors to become metastatic, they must degrade their surrounding extracellular matrix components, degrade basement

membranes to gain access to the bloodstream or lymph system, and repeat this process in reverse fashion to settle in a secondary host site [3-6]. All of these processes rely upon what now appears to be a synchronized protease cascade. In addition, tumor cells
5 use the power of proteases to activate growth and angiogenic factors that allow the tumor to grow progressively [1]. Therefore, much research has been aimed at the identification of tumor-associated proteases and the inhibition of these enzymes for therapeutic means. More importantly, the secreted nature and/or
10 high level expression of many of these proteases allows for their detection at aberrant levels in patient serum, e.g. the prostate-specific antigen (PSA), which allows for early diagnosis of prostate cancer [7].

Proteases have been associated directly with tumor
15 growth, shedding of tumor cells and invasion of target organs. Individual classes of proteases are involved in, but not limited to (1) the digestion of stroma surrounding the initial tumor area, (2) the digestion of the cellular adhesion molecules to allow dissociation of tumor cells; and (3) the invasion of the basement
20 membrane for metastatic growth and the activation of both tumor growth factors and angiogenic factors.

For many forms of cancer, diagnosis and treatment has improved dramatically in the last 10 years. However, the five year survival rate for ovarian cancer remains below 50% due in
25 large part to the vague symptoms which allow for progression of the disease to an advanced stage prior to diagnosis [8]. Although the exploitation of the CA125 antigen has been useful as a marker for monitoring recurrence of ovarian cancer, it has not proven to be an ideal marker for early diagnosis. Therefore, new markers

that may be secreted or released from cells and which are highly expressed by ovarian tumors could provide a useful tool for the early diagnosis and for therapeutic intervention in patients with ovarian carcinoma.

5 The prior art is deficient in the lack of the complete identification of the proteases overexpressed in carcinoma, therefore, deficient in the lack of a tumor marker useful as an indicator of early disease, particularly for ovarian cancers. Specifically, TADG-12, a transmembrane serine protease, has not
10 been previously identified in either nucleic acid or protein form. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

15

 The present invention discloses TADG-12, a new member of the Tumor Associated Differentially-Expressed Gene (TADG) family, and a variant splicing form of TADG-12 (TADG-12V) that could lead to a truncated protein product. TADG-12 is a
20 transmembrane serine protease overexpressed in ovarian carcinoma. The entire cDNA of TADG-12 has been identified (SEQ ID No. 1). This sequence encodes a putative protein of 454 amino acids (SEQ ID No. 2) which includes a potential transmembrane domain, an LDL receptor like domain, a scavenger receptor
25 cysteine rich domain, and a serine protease domain. These features imply that TADG-12 is expressed at the cell surface, and it may be used as a molecular target for therapy or a diagnostic marker.

In one embodiment of the present invention, there is provided a DNA fragment encoding a TADG-12 protein selected from the group consisting of: (a) an isolated DNA fragment which encodes a TADG-12 protein; (b) an isolated DNA fragment which hybridizes to isolated DNA fragment of (a) above and which encodes a TADG-12 protein; and (c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Specifically, the DNA fragment has a sequence shown in SEQ ID No. 1 or SEQ ID No. 3.

In another embodiment of the present invention, there is provided a vector/host cell capable of expressing the DNA of the present invention.

In yet another embodiment of the present invention, there is provided an isolated and purified TADG-12 protein encoded by DNA selected from the group consisting of: (a) isolated DNA which encodes a TADG-12 protein; (b) isolated DNA which hybridizes to isolated DNA of (a) above and which encodes a TADG-12 protein; and (c) isolated DNA differing from the isolated DNAs of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Specifically, the TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

In still yet another embodiment of the present invention, there is provided a method for detecting expression of a TADG-12 protein, comprising the steps of: (a) contacting mRNA obtained from the cell with the labeled hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

The present invention further provides methods for diagnosing a cancer or other malignant hyperplasia by detecting the TADG-12 protein or mRNA disclosed herein.

In still another embodiment of the present invention, there is provided a method of inhibiting expression of endogenous TADG-12 mRNA in a cell by introducing a vector into the cell, wherein the vector comprises a DNA fragment of TADG-12 in opposite orientation operably linked to elements necessary for expression.

In still yet another embodiment of the present invention, there is provided a method of inhibiting expression of a TADG-12 protein in a cell by introducing an antibody directed against a TADG-12 protein or fragment thereof.

In still yet another embodiment of the present invention, there is provided a method of targeted therapy by administering a compound having a targeting moiety specific for a TADG-12 protein and a therapeutic moiety. Specifically, the TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

The present invention still further provides a method of vaccinating an individual against TADG-12 by inoculating the individual with a TADG-12 protein or fragment thereof. Specifically, the TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4. The TADG-12 fragment includes the truncated form of TADG-12V peptide having a sequence shown in SEQ ID No. 8, and a 9-residue up to 12-residue fragment of TADG-12 protein.

In yet another embodiment of the present invention, there is provided an immunogenic composition, comprising an

immunogenic fragment of a TADG-12 protein and an appropriate adjuvant. The TADG-12 fragment includes the truncated form of TADG-12V peptide having a sequence shown in SEQ ID No. 8, and a 9-residue up to 12-residue fragment of TADG-12 protein.

In yet another embodiment of the present invention, there is provided use in
 5 preparation of a medicament to inhibit expression of endogenous TADG-12 mRNA in a cell of a vector comprising a TADG-12 DNA fragment described herein in opposite orientation operably linked to elements necessary for expression, such that expression of said vector in said cell produces TADG-12 antisense mRNA.

In yet another embodiment of the present invention, there is provided use of an
 10 antibody directed against a TADG-12 protein described herein or a fragment thereof in the preparation of a medicament to inhibit expression of said TADG-12 protein.

In yet another embodiment of the present invention, there is provided use in the preparation of a medicament for targeted therapy of an individual of a targeting moiety specific for a TADG-12 protein with an amino acid sequence of SEQ ID NO: 2 or SEQ ID
 15 NO: 4, combined with and a therapeutic moiety which is a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant or a cytotoxic agent combined.

In yet another embodiment of the present invention, there is provided an immunogenic composition, comprising a TADG-12 protein of SEQ ID NO: 2 or SEQ ID NO: 4 or an immunogenic fragment of TADG-12 protein that has an amino acid sequence
 20 shown in SEQ ID NOS. 8, 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 or 136 and an appropriate adjuvant.

In yet another embodiment of the present invention, there is provided use of the immunogenic composition described herein in the preparation of a medicament to vaccinate an individual against TADG-12.

25 In yet another embodiment of the present invention, there is provided use of the immunogenic composition described herein to vaccinate an individual against TADG-12.

In yet another embodiment of the present invention, there is provided use to inhibit expression of endogenous TADG-12 mRNA in a cell of a vector comprising a TADG-12 DNA fragment described herein in opposite orientation operably linked to elements
 30 necessary for expression, such that expression of said vector in said cell produces TADG-12 antisense mRNA.

In yet another embodiment of the present invention, there is provided use of an antibody directed against a TADG-12 protein described herein or a fragment thereof to inhibit expression of said TADG-12 protein.

5 In yet another embodiment of the present invention, there is provided use for targeted therapy of an individual of a targeting moiety specific for a TADG-12 protein with an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, combined with a therapeutic moiety which is a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant or a cytotoxic agent.

10 BRIEF DESCRIPTION OF THE DRAWINGS

15 So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention briefly summarized above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that 20 the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

25 **Figure 1A** shows that the expected PCR product of approximately 180 bp and the unexpected PCR product of approximately 300 bp using the redundant serine protease primers were not amplified from normal ovary cDNA (Lane 1) but were found in abundance from ovarian tumor cDNA (Lane 2). The primer sequences for the PCR reactions are indicated by horizontal 30 arrows. **Figure 1B** shows that TADG-12 was subcloned from the 180 bp band while the larger 300 bp band was designated TADG-

12V. The sequences were found to overlap for 180 bp (SEQ ID No. 5 for nucleotide sequence, SEQ ID No. 6 for deduced amino acid sequence) with the 300 bp TADG-12V (SEQ ID No. 7 for nucleotide sequence, SEQ ID No. 8 for deduced amino acid sequence) having an additional insert of 133 bases. This insertion (vertical arrow) leads to a frame shift, which causes the TADG-12V transcript to potentially produce a truncated form of TADG-12 with a variant amino acid sequence.

Figure 2 shows that Northern blot analysis for TADG-12 revealed three transcripts of 2.4, 1.6 and 0.7 kilobases. These transcripts were found at significant levels in ovarian tumors and cancer cell lines, but the transcripts were found only at low levels in normal ovary.

Figure 3 shows an RNA dot blot (CLONTECH) probed for TADG-12. The transcript was detectable (at background levels) in all 50 of the human tissues represented with the greatest abundance of transcript in the heart. Putamen, amygdala, kidney, liver, small intestine, skeletal muscle, and adrenal gland were also found to have intermediate levels of TADG-12 transcript.

Figure 4 shows the entire cDNA sequence for TADG-12 (SEQ ID No. 1) with its predicted open reading frame of 454 amino acids (SEQ ID No. 2). Within the nucleotide sequence, the Kozak's consensus sequence for the initiation of translation and the poly-adenylation signal are underlined. In the protein sequence, a potential transmembrane domain is boxed. The LDLR-A domain is underlined with a solid line. The SRCR domain is underlined with a broken line. The residues of the catalytic triad of the serine protease domain are circled, and the beginning of the

catalytic domain is marked with an arrow designated as a potential proteolytic cleavage site. The * represents the stop codon that terminates translation.

Figure 5A shows the 35 amino acid LDLR-A domain of TADG-12 (SEQ ID No. 13) aligned with other LDLR-A motifs from the serine protease TMPRSS2 (U75329, SEQ ID No. 14), the complement subunit C8 (P07358, SEQ ID No. 9), two LDLR-A domains of the glycoprotein GP300 (P98164, SEQ ID Nos. 11-12), and the serine protease matriptase (AF118224, SEQ ID No. 10). TADG-12 has its highest similarity with the other serine proteases for which it is 54% similar to TMPRSS2 and 53% similar to matriptase. The highly conserved cysteine residues are shown in bold type. **Figure 5B** shows the SRCR domain of TADG-12 (SEQ ID No. 17) aligned with other domain family members including the human macrophage scavenger receptor (P21757, SEQ ID No. 16), human enterokinase (P98073, SEQ ID No. 19), bovine enterokinase (P21758, SEQ ID No. 15), and the serine protease TMPRSS2 (SEQ ID No. 18). Again, TADG-12 shows its highest similarity within this region to the protease TMPRSS2 at 43%. **Figure 5C** shows the protease domain of TADG-12 (SEQ ID No. 23) in alignment with other human serine proteases including protease M (U62801, SEQ ID No. 20), trypsinogen I (P07477, SEQ ID No. 21), plasma kallikrein (P03952, SEQ ID No. 22), hepsin (P05981, SEQ ID No. 25), and TMPRSS2 (SEQ ID No. 24). Cons represents the consensus sequence for each alignment.

Figure 6 shows semi-quantitative PCR analysis that was performed for TADG-12 (upper panel) and TADG-12V (lower panel). The amplification of TADG-12 or TADG-12V was performed in parallel with PCR amplification of β -tubulin product

as an internal control. The TADG-12 transcript was found to be overexpressed in 41 of 55 carcinomas. The TADG-12V transcript was found to be overexpressed in 8 of 22 carcinomas examined. Note that the samples in the upper panel are not necessarily the same as the samples in the lower panel.

Figure 7 shows immunohistochemical staining of normal ovary and ovarian tumors which were performed using a polyclonal rabbit antibody developed to a TADG-12 specific peptide. No significant staining was detected in normal ovary (**Figure 7A**). Strong positive staining was observed in 22 of 29 carcinomas examined. **Figures 7B** and **7C** represent a serous and mucinous carcinoma, respectively. Both show diffuse staining throughout the cytoplasm of tumor cells while stromal cells remain relatively unstained.

Figure 8 is a model to demonstrate the progression of TADG-12 within a cellular context. In normal circumstances, the TADG-12 transcript is appropriately spliced and the resulting protein is capable of being expressed at the cell surface where the protease may be cleaved to an active form. The role of the remaining ligand binding domains has not yet been determined, but one can envision their potential to bind other molecules for activation, internalization or both. The TADG-12V transcript, which occurs in some tumors, may be the result of mutation and/or poor mRNA processing may be capable of producing a truncated form of TADG-12 that does not have a functional protease domain. In addition, this truncated product may present a novel epitope at the surface of tumor cells.

DETAILED DESCRIPTION OF THE INVENTION

To examine the serine proteases expressed by ovarian cancers, a PCR based differential display technique was employed
5 utilizing redundant PCR primers designed to the most highly conserved amino acids in these proteins [9]. As a result, a novel cell-surface, multi-domain serine protease, named Tumor Associated Differentially-expressed Gene-12 (TADG-12) was identified. TADG-12 appears to be overexpressed in many ovarian
10 tumors. The extracellular nature of TADG-12 may render tumors susceptible to detection via a TADG-12 specific assay. In addition, a splicing variant of TADG-12, named TADG-12V, was detected at elevated levels in 35% of the tumors that were examined. TADG-12V encodes a truncated form of TADG-12 with an altered amino
15 acid sequence that may be a unique tumor specific target for future therapeutic approaches.

The TADG-12 cDNA is 2413 base pairs long (SEQ ID No. 1) encoding a 454 amino acid protein (SEQ ID No. 2). A variant form, TADG-12V (SEQ ID No. 3), encodes a 294 amino acid protein
20 (SEQ ID No. 4). The availability of the TADG-12 and/or TADG-12V gene opens the way for a number studies that can lead to various applications. For example, the TADG-12 and/or TADG-12V gene can be used as a diagnostic or therapeutic target in ovarian carcinoma and other carcinomas including breast, prostate, lung
25 and colon.

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. NH₂ refers to the free amino group present at

the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J Biol. Chem.*, 243:3552-59 (1969), abbreviations for amino acid residues are
5 known in the art.

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
10 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
15 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.

20 A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or 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.
25 Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure 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 "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

5 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 determined by a start codon at the 5' (amino) terminus and a translation stop
10 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
15 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.

20 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
25 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 contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

5 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
10 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
15 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.

20 The term "oligonucleotide", as used herein in referring to the probe of the present invention, 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.

25 The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which

is complementary to a nucleic acid strand, is induced, 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, source of primer and use the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. 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 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 or hybridize therewith and thereby form the template for the synthesis of the extension product.

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.

5 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
10 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.

15 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
20 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,
25 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. In another example, coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where
5 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
10 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
15 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
20 isotope may be selected from ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{125}I , ^{131}I , and ^{186}Re .

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques.
25 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.

5 A particular assay system developed and utilized in the art is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantity of both the label after which binding studies are conducted to determine the
10 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
15 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
20 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
25 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 TADG-12 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 huma TADG-12 protein of the present invention for purposes of prokaryote transformation. Prokaryotic hosts may include *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. Eukaryotic hosts include yeasts such as *Pichia pastoris*, mammalian cells and insect cells.

In general, expression vectors containing promoter sequences which facilitate the efficient transcription of the inserted DNA fragment are used in connection with the 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.

The invention includes a substantially pure DNA encoding a TADG-12 protein, a strand of which DNA will hybridize at high stringency to a probe containing a sequence of at least 15 consecutive nucleotides of the sequence shown in SEQ ID No. 1 or SEQ ID No. 3. The protein encoded by the DNA of this invention may share at least 80% sequence identity (preferably 85%, more preferably 90%, and most preferably 95%) with the amino acids

listed in SEQ ID No. 2 or SEQ ID No. 4. More preferably, the DNA includes the coding sequence of the nucleotides of Figure 4 (SEQ ID No. 1), or a degenerate variant of such a sequence.

5 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 Figure 4 (SEQ ID No. 1) or the complement
10 thereof. Such a probe is useful for detecting expression of TADG-12 in a human cell by a method including the steps of (a) contacting mRNA obtained from the cell with the labeled hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

15 This invention also includes a substantially pure DNA containing a sequence of at least 15 consecutive nucleotides (preferably 20, more preferably 30, even more preferably 50, and most preferably all) of the region from nucleotides 1 to 2413 of the nucleotides listed in SEQ ID No. 1, or of the region from
20 nucleotides 1 to 2544 of the nucleotides listed in SEQ ID No. 3. The present invention also comprises antisense oligonucleotides directed against this novel DNA. Given the teachings of the present invention, a person having ordinary skill in this art would readily be able to develop antisense oligonucleotides directed
25 against this DNA.

By "high stringency" is meant DNA hybridization and wash conditions characterized by high temperature and low salt concentration, e.g., wash conditions of 65°C at a salt concentration of approximately 0.1 x SSC, or the functional equivalent thereof.

For example, high stringency conditions may include hybridization at about 42°C in the presence of about 50% formamide; a first wash at about 65°C with about 2 x SSC containing 1% SDS; followed by a second wash at about 65°C with about 0.1 x SSC.

5 By "substantially pure DNA" is meant 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
10 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
15 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 shown in SEQ ID No. 3 which encodes an
20 alternative splice variant of TADG-12 (TADG-12V).

The DNA may have at least about 70% sequence identity to the coding sequence of the nucleotides listed in SEQ ID No. 1 or SEQ ID No. 3, preferably at least 75% (e.g. at least 80%); and most preferably at least 90%. The identity between two
25 sequences is a direct function of the number of matching or identical positions. When a subunit 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, University of Wisconsin Biotechnology Center, 10 1710 University Avenue, Madison, WI 53705).

The present invention comprises a vector comprising a DNA sequence which encodes a human TADG-12 protein and the vector is capable of replication in a host which comprises, in operable linkage: a) an origin of replication; b) a promoter; and c) 15 a DNA sequence coding for said protein. Preferably, the vector of the present invention contains a portion of the DNA sequence shown in SEQ ID No. 1 or SEQ ID No. 3. A "vector" may be defined as a replicable nucleic acid construct, e.g., a plasmid or viral nucleic acid. Vectors may be used to amplify and/or express 20 nucleic acid encoding a TADG-12 protein. 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 25 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, the 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 the 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.

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 is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight. A substantially pure TADG-12 protein may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding an TADG-12 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 TADG-12, 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
5 eukaryotic proteins synthesized in *E. coli*, other prokaryotes, or any other organism in which they do not naturally occur.

In addition to substantially full-length proteins, the invention also includes fragments (e.g., antigenic fragments) of the TADG-12 protein. As used herein, "fragment," as applied to a
10 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 TADG-12 protein can be generated by methods known to those skilled in the art, e.g., by enzymatic digestion of naturally
15 occurring or recombinant TADG-12 protein, by recombinant DNA techniques using an expression vector that encodes a defined fragment of TADG-12, or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of TADG-12 (e.g., binding to an antibody specific for TADG-12) can be assessed by
20 methods described herein. Purified TADG-12 or antigenic fragments of TADG-12 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 are polyclonal antisera
25 generated by using TADG-12 or a fragment of TADG-12 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

TADG-12 cDNA clones, and to distinguish them from known cDNA clones.

Further included in this invention are TADG-12 proteins which are encoded at least in part by portions of SEQ ID No. 1 or SEQ ID No. 3, e.g., products of alternative mRNA splicing or alternative protein processing events, or in which a section of TADG-12 sequence has been deleted. The fragment, or the intact TADG-12 polypeptide, may be covalently linked to another polypeptide, e.g. 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 TADG-12. The invention encompasses not only an intact monoclonal antibody, but also an immunologically-active antibody fragment, e.g., a Fab or (Fab)₂ fragment; an engineered single chain Fv molecule; or a chimeric molecule, e.g., an antibody which contains the binding specificity of one antibody, e.g., of murine origin, and the remaining portions of another antibody, e.g., of human origin.

In one embodiment, the antibody, or a fragment thereof, may be linked to a toxin or to a detectable label, e.g. a radioactive label, non-radioactive isotopic label, fluorescent label, chemiluminescent label, paramagnetic label, enzyme label, or colorimetric label. Examples of suitable toxins include diphtheria toxin, *Pseudomonas* exotoxin A, ricin, and cholera toxin. Examples of suitable enzyme labels include malate hydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease,

urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholinesterase, etc. Examples of suitable radioisotopic labels include ^3H , ^{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 to 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
5 TADG-12 protein in a biological sample, which includes the steps of contacting the sample with the labeled antibody, e.g., radioactively tagged antibody specific for TADG-12, and determining whether the antibody binds to a component of the sample.

10 As described herein, the invention provides a number of diagnostic advantages and uses. For example, the TADG-12 protein disclosed in the present invention is useful in diagnosing cancer in different tissues since this protein is highly overexpressed in tumor cells. Antibodies (or antigen-binding
15 fragments thereof) which bind to an epitope specific for TADG-12, are useful in a method of detecting TADG-12 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
20 having cancer, contacting the sample with a labeled antibody (e.g., radioactively tagged antibody) specific for TADG-12, and detecting the TADG-12 protein using standard immunoassay techniques such as an ELISA. Antibody binding to the biological sample indicates that the sample contains a component which specifically
25 binds to an epitope within TADG-12.

Likewise, a standard Northern blot assay can be used to ascertain the relative amounts of TADG-12 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 TADG-12 cDNA, either containing the full-length, single stranded DNA having a sequence complementary to SEQ ID No. 1 or SEQ ID No. 3, or a
5 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.

10 Antibodies to the TADG-12 protein can be used in an immunoassay to detect increased levels of TADG-12 protein expression in tissues suspected of neoplastic transformation. These same uses can be achieved with Northern blot assays and analyses.

15 The present invention is directed to DNA fragment encoding a TADG-12 protein selected from the group consisting of:
(a) an isolated DNA fragment which encodes a TADG-12 protein;
(b) an isolated DNA fragment which hybridizes to isolated DNA fragment of (a) above and which encodes a TADG-12 protein; and
20 (c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Preferably, the DNA has the sequence shown in SEQ ID No. 1 or SEQ ID No. 3. More preferably, the DNA encodes a TADG-
25 12 protein having the amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

The present invention is also directed to a vector and/or a host cell capable of expressing the DNA of the present invention. Preferably, the vector contains DNA encoding a TADG-

12 protein having the amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4. Representative host cells include bacterial cells, yeast cells, mammalian cells and insect cells.

5 The present invention is also directed to an isolated and purified TADG-12 protein coded for by DNA selected from the group consisting of: (a) isolated DNA which encodes a TADG-12 protein; (b) isolated DNA which hybridizes to isolated DNA of (a) above and which encodes a TADG-12 protein; and (c) isolated DNA differing from the isolated DNAs of (a) and (b) above in codon
10 sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Preferably, the isolated and purified TADG-12 protein has the amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

The present invention is also directed to a method of
15 detecting expression of the TADG-12 protein described herein, comprising the steps of: (a) contacting mRNA obtained from the cell with the labeled hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

A number of potential applications are possible for the
20 TADG-12 gene and gene product including the truncated product TADG-12V.

In one embodiment of the present invention, there is provided a method for diagnosing a cancer by detecting a TADG-12 protein in a biological sample, wherein the presence or absence
25 of a TADG-12 protein indicates the presence or absence of a cancer. Preferably, the biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells. Still preferably, the detection of TADG-12 protein 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. Such method is used for detecting an ovarian cancer, breast cancer, lung cancer, colon cancer, prostate cancer
5 and other cancers in which TADG-12 is overexpressed.

In another embodiment of the present invention, there is provided a method for detecting malignant hyperplasia by detecting a TADG-12 protein or TADG-12 mRNA in a biological sample. Further by comprising the TADG-12 protein or TADG-12
10 mRNA to reference information, a diagnosis or a treatment can be provided. Preferably, PCR amplification is used for detecting TADG-12 mRNA, wherein the primers utilized are selected from the group consisting of SEQ ID Nos. 28-31. Still preferably, detection of a TADG-12 protein is by immunoaffinity to an
15 antibody directed against a TADG-12 protein.

In still another embodiment of the present invention, there is provided a method of inhibiting expression of endogenous TADG-12 mRNA in a cell by introducing a vector comprising a DNA fragment of TADG-12 in opposite orientation operably linked to
20 elements necessary for expression. As a result, the vector produces TADG-12 antisense mRNA in the cell, which hybridizes to endogenous TADG-12 mRNA, thereby inhibiting expression of endogenous TADG-12 mRNA.

In still yet another embodiment of the present
25 invention, there is provided a method of inhibiting expression of a TADG-12 protein by introducing an antibody directed against a TADG-12 protein or fragment thereof. As a result, the binding of the antibody to the TADG-12 protein or fragment thereof inhibits the expression of the TADG-12 protein.

TADG-12 gene products including the truncated form can be used for targeted therapy. Specifically, a compound having a targeting moiety specific for a TADG-12 protein and a therapeutic moiety is administered to an individual in need of such treatment. Preferably, the targeting moiety is selected from the group consisting of an antibody directed against a TADG-12 protein and a ligand or ligand binding domain that binds a TADG-12 protein. The TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4. Still preferably, the therapeutic moiety is selected from the group consisting of a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant and a cytotoxic agent. Such method can be used for treating an individual having a disease selected from the group consisting of ovarian cancer, lung cancer, prostate cancer, colon cancer and other cancers in which TADG-12 is overexpressed.

In yet another embodiment of the present invention, there is provided a method of vaccinating, or producing an immune response in, an individual against TADG-12 by inoculating the individual with a TADG-12 protein or fragment thereof. Specifically, the TADG-12 protein or fragment thereof lacks TADG-12 activity, and the inoculation elicits an immune response in the individual, thereby vaccinating the individual against TADG-12. Preferably, the individual has a cancer, is suspected of having a cancer or is at risk of getting a cancer. Still preferably, TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4, while TADG-12 fragment has a sequence shown in SEQ ID No. 8, or is a 9-residue fragment up to a 20-residue fragment. Examples of 9-residue fragment are shown in SEQ ID Nos. 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 and 136.

In still yet another embodiment of the present invention, there is provided an immunogenic composition, comprising an immunogenic fragment of a TADG-12 protein and an appropriate adjuvant. Preferably, the immunogenic fragment
5 of the TADG-12 protein has a sequence shown in SEQ ID No. 8, or is a 9-residue fragment up to a 20-residue fragment. Examples of 9-residue fragment are shown in SEQ ID Nos. 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 and 136.

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

EXAMPLE 1

Tissue collection and storage

15 Upon patient hysterectomy, bilateral salpingo-oophorectomy, or surgical removal of neoplastic tissue, the specimen is retrieved and placed on ice. The specimen was then taken to the resident pathologist for isolation and identification of specific tissue samples. Finally, the sample was frozen in liquid
20 nitrogen, logged into the laboratory record and stored at -80°C. Additional specimens were frequently obtained from the Cooperative Human Tissue Network (CHTN). These samples were prepared by the CHTN and shipped on dry ice. Upon arrival, these specimens were logged into the laboratory record and stored at -
25 80°C.

EXAMPLE 2

mRNA Extraction and cDNA Synthesis

Sixty-nine ovarian tumors (4 benign tumors, 10 low malignant potential tumors and 55 carcinomas) and 10 normal

ovaries were obtained from surgical specimens and frozen in liquid nitrogen. The human ovarian carcinoma cell lines SW 626 and Caov 3, the human breast carcinoma cell lines MDA-MB-231 and MDA-MB-435S were purchased from the American Type Culture Collection (Rockville, MD). Cells were cultured to sub-confluency in Dulbecco's modified Eagle's medium, supplemented with 10% (v/v) fetal bovine serum and antibiotics.

Extraction of mRNA and cDNA synthesis were carried out by the methods described previously [14-16]. mRNA was isolated by using a RiboSep* mRNA isolation kit (Becton Dickinson Labware). In this procedure, poly A+ mRNA was isolated directly from the tissue lysate using the affinity chromatography media oligo(dT) cellulose. cDNA was synthesized with 5.0 µg of mRNA by random hexamer priming using 1st strand cDNA synthesis kit (CLONTECH).

EXAMPLE 3

PCR with Redundant Primers and Cloning of TADG-12 cDNA

Redundant primers, forward 5'-
 20 TGGGTIGTIACIGCIGCICA(CT)TG -3' (SEQ ID No. 26) and reverse 5'-
 A(AG)IA(AG)IGCIATITCITTICC-3' (SEQ ID No. 27), for the
 consensus sequences of amino acids surrounding the catalytic
 triad for serine proteases were used to compare the PCR products
 from normal and carcinoma cDNAs. The appropriate bands were
 25 ligated into Promega T-vector plasmid and the ligation product
 was used to transform JM109 cells (Promega) grown on selection
 media. After selection of individual colonies, they were cultured
 and plasmid DNA was isolated by means of the Wizard miniprep
 DNA purification system (Promega). Nucleotide sequencing was

5 The original TADG-12 subclone was randomly labeled
and used as a probe to screen an ovarian tumor cDNA library by
standard hybridization techniques [11,15]. The library was
constructed in λ ZAP using mRNA isolated from the tumor cells of a
stage III/grade III ovarian adenocarcinoma patient. Three
10 overlapping clones were obtained which spanned 2315
nucleotides. The final 99 nucleotides encoding the most 3'
sequence including the poly A tail was identified by homology
with clones available in the GenBank* EST database.

15 EXAMPLE 4
Quantitative PCR

The mRNA overexpression of TADG-12 was determined using a quantitative PCR. Quantitative PCR was performed according to the procedure as previously reported [16].

20 Oligonucleotide primers were used for: TADG-12, forward 5'-GAAACATGTCCTTGCTCTCG-3' (SEQ ID No. 28) and reverse 5'-ACTAACTTCCACAGCCTCCT-3' (SEQ ID No. 29); the variant TADG-12, forward 5'-TCCAGGTGGGTCTAGTTTCC-3' (SEQ ID No. 30), reverse 5'-CTCTTTGGCTTGTACTTGCT-3' (SEQ ID No. 31); β -tubulin, forward

25 5'-CGCATCAACGTGTACTACAA-3' (SEQ ID No. 32) and reverse 5'-TACGAGCTGGTGGACTGAGA-3' (SEQ ID No. 33). β -tubulin was utilized as an internal control. The PCR reaction mixture consists of cDNA derived from 50 ng of mRNA, 5 pmol of sense and antisense primers for both the TADG-12 gene and the β -tubulin

gene, 200 μmol of dNTPs, 5 μCi of $\alpha\text{-}^{32}\text{PdCTP}$ and 0.25 unit 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
5 Cycler (Perkin-Elmer Cetus). Each cycle of PCR included 30 seconds of denaturation at 94°C, 30 seconds of annealing at 60°C and 30 seconds of extension at 72°C. The PCR products were separated on 2% agarose gels and the radioactivity of each PCR product was determined by using a Phospho Imager (Molecular
10 Dynamics). The present study used the expression ratio (TADG-12/ β -tubulin) as measured by phosphoimager to evaluate gene expression and defined the value at mean + 2SD of normal ovary as the cut-off value to determine overexpression. The student's *t* test was used for comparison of the mean values of normal ovary
15 and tumors.

EXAMPLE 5

Sequencing of TADG-12/TADG-12V

Utilizing a plasmid specific primer near the cloning
20 site, sequencing reactions were carried out using PRISM™ Ready Reaction Dye Deoxy™ terminators (Applied Biosystems cat# 401384) according to the manufacturer's instructions. Residual dye terminators were removed from the completed sequencing reaction using a Centri-sep™ spin column (Princeton Separation
25 cat.# CS-901). An Applied Biosystems Model 373A DNA Sequencing System was available and was used for sequence analysis.

EXAMPLE 6

Antibody Production

Polyclonal rabbit antibodies were generated by immunization of white New Zealand rabbits with a poly-lysine
5 linked multiple antigen peptide derived from the TADG-12
carboxy-terminal protein sequence $\text{NH}_2\text{-WIHEQMERDLKT-COOH}$
(WIHEQMERDLKT, SEQ ID No. 34). This peptide is present in full
length TADG-12, but not TADG-12V. Rabbits were immunized
with approximately 100 μg of peptide emulsified in Ribi adjuvant.
10 Subsequent boost immunizations were carried out at 3 and 6
weeks, and rabbit serum was isolated 10 days after the boost
inoculations. Sera were tested by dot blot analysis to determine
affinity for the TADG-12 specific peptide. Rabbit pre-immune
serum was used as a negative control.

15

EXAMPLE 7

Northern Blot Analysis

10 μg of mRNA were loaded onto a 1% formaldehyde-
agarose gel, electrophoresed and blotted on a Hybond^{*}-N+ nylon
20 membrane (Amersham). ^{32}P -labeled cDNA probes were made by
Prime-a-Gene Labeling System (Promega). The PCR products
amplified by the same primers as above were used for probes.
The blots were prehybridized for 30 min and hybridized for 60
min at 68°C with ^{32}P -labeled cDNA probe in ExpressHyb
25 Hybridization Solution (CLONTECH). Control hybridization to
determine relative gel loading was performed with the β -tubulin
probe.

* Trade-mark

Normal human tissues; spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocyte, and normal human fetal tissues; brain, lung, liver and kidney (Human Multiple Tissue Northern Blot; CLONTECH) were also examined by
5 same hybridization procedure.

EXAMPLE 8

Immunohistochemistry

Immunohistochemical staining was performed using a
10 Vectastain Elite ABC Kit (Vector). Formalin fixed and paraffin embedded specimens were routinely deparaffinized and processed using microwave heat treatment in 0.01 M sodium citrate buffer (pH 6.0). The specimens were incubated with normal goat serum in a moist chamber for 30 minutes. TADG-12 peptide antibody
15 was allowed to incubate with the specimens in a moisture chamber for 1 hour. Excess antibody was washed away with phosphate buffered saline. After incubation with biotinylated anti-rabbit IgG for 30 minutes, the sections were then incubated with ABC reagent (Vector) for 30 minutes. The final products
20 were visualized using the AEC substrate system (DAKO) and sections were counterstained with hematoxylin before mounting. Negative controls were performed by using normal serum instead of the primary antibody.

25

EXAMPLE 9

Isolation of Catalytic Domain Subclones of TADG-12 and TADG-12 Variant

To identify serine proteases that are expressed in ovarian tumors, redundant PCR primers designed to the conserved

regions of the catalytic triad of these enzymes were employed. A sense primer designed to the region surrounding the conserved histidine and an anti-sense primer designed to the region surrounding the conserved aspartate were used in PCR reactions with either normal ovary or ovarian tumor cDNA as template. In the reaction with ovarian tumor cDNA, a strong product band of the expected size of approximately 180 bp was observed as well as an unexpected PCR product of approximately 300 bp which showed strong expression in some ovarian tumor cDNA's (Figure 1A). Both of these PCR products were subcloned and sequenced. The sequence of the subclones from the 180bp band (SEQ ID No. 5) was found to be homologous to the sequence identified in the larger, unexpected band (SEQ ID No. 7) except that the larger band had an additional insert of 133 nucleotides (Figure 1B). The smaller product of the appropriate size encoded for a protein sequence (SEQ ID No. 6) homologous to other known proteases while the sequence with the insertion (SEQ ID No. 8) encoded for a frame shift from the serine protease catalytic domain and a subsequent premature translational stop codon. TADG-12 variants from four individual tumors were also subcloned and sequenced. It was found that the sequence and insert to be identical. The genomic sequences for these cDNA derived clones were amplified by PCR, examined and found to contain potential AG/GT splice sites that would allow for the variant transcript production.

25

EXAMPLE 10

Northern Blot Analysis of TADG-12 Expression

To examine transcript size and tissue distribution, the catalytic domain subclone was randomly labeled and used to

probe Northern blots representing normal ovarian tissue, ovarian tumors and the cancer cell lines SW626, CAOV3, HeLa, MD-MBA-435S and MD-MBA-231 (Figure 2). Three transcripts of 2.4, 1.6 and 0.7 kilobases were observed. In blots of normal and ovary tumor the smallest transcript size 0.7 kb was lowly expressed in normal ovary while all transcripts (2.4, 1.6 and 0.7 kb) were abundantly present in serous carcinoma. In addition, Northern blots representing the normal human tissues spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocyte, and normal human fetal tissues of brain, lung, liver and kidney were examined. The same three transcripts were found to be expressed weakly in all of these tissues (data not shown). A human β -tubulin specific probe was utilized as a control for relative sample loading. In addition, an RNA dot blot was probed representing 50 human tissues and determined that this clone is weakly expressed in all tissues represented (Figure 3). It was found most prominently in heart, with intermediate levels in putamen, amygdala, kidney, liver, small intestine, skeletal muscle, and adrenal gland.

20

EXAMPLE 11

Sequencing and Characterization of TADG-12

An ovarian tumor cDNA library constructed in λ ZAP was screened by standard hybridization techniques using the catalytic domain subclone as a probe. Two clones that overlapped with the probe were identified and sequenced and found to represent 2316 nucleotides. The 97 nucleotides at the 3' end of the transcript including the poly-adenylation signal and the poly (A) tail were identified by homology with clones available in

25

GenBank's EST database. This brought the total size of the transcript to 2413 bases (SEQ ID No. 1, Figure 4). Subsequent screening of GenBank's Genomic Database revealed that TADG-12 is homologous to a cosmid from chromosome 17. This cosmid has
5 the accession number AC015555.

The identified cDNA includes an open reading frame that would produce a predicted protein of 454 amino acids (SEQ ID No. 2), named Tumor Associated Differentially-Expressed Gene 12 (TADG-12). The sequence has been submitted to the GenBank
10 database and granted the accession # AF201380. Using homology alignment programs, this protein contains several domains including an amino-terminal cytoplasmic domain, a potential Type II transmembrane domain followed by a low-density lipoprotein receptor-like class A domain (LDLR-A), a scavenger receptor
15 cysteine rich domain (SRCR), and an extracellular serine protease domain.

As predicted by the TMPred program, TADG-12 contains a highly hydrophobic stretch of amino acids that could serve as a potential transmembrane domain, which would retain the amino
20 terminus of the protein within the cytoplasm and expose the ligand binding domains and protease domain to the extracellular space. This general structure is consistent with other known transmembrane proteases including hepsin [17], and TMPRSS2 [18], and TADG-12 is particularly similar in structure to the
25 TMPRSS2 protease.

The LDLR-A domain of TADG-12 is represented by the sequence from amino acid 74 to 108 (SEQ ID No. 13). The LDLR-A domain was originally identified within the LDL Receptor [19] as a series of repeated sequences of approximately 40 amino acids,

which contained 6 invariant cysteine residues and highly conserved aspartate and glutamate residues. Since that initial identification, a host of other genes have been identified which contain motifs homologous to this domain [20]. Several proteases
5 have been identified which contain LDLR-A motifs including matriptase, TMPRSS2 and several complement components. A comparison of TADG-12 with other known LDLR-A domains is shown in Figure 5A. The similarity of these sequences range from 44 to 54% of similar or identical amino acids.

10 In addition to the LDLR-A domain, TADG-12 contains another extracellular ligand binding domain with homology to the group A SRCR family. This family of protein domains typically is defined by the conservation of 6 cysteine residues within a sequence of approximately 100 amino acids [23]. The SRCR
15 domain of TADG-12 is encoded by amino acids 109 to 206 (SEQ ID No. 17), and this domain was aligned with other SRCR domains and found to have between 36 and 43% similarity (Figure 5B). However, TADG-12 only has 4 of the 6 conserved cysteine residues. This is similar to the SRCR domain found in the protease
20 TMPRSS2.

The TADG-12 protein also includes a serine protease domain of the trypsin family of proteases. An alignment of the catalytic domain of TADG-12 with other known proteases is shown in Figure 5C. The similarity among these sequence ranges from 48
25 to 55%, and TADG-12 is most similar to the serine protease TMPRSS2 which also contains a transmembrane domain, LDLR-A domain and an SRCR domain. There is a conserved amino acid motif (RIVGG) downstream from the SRCR domain that is a potential cleavage/activation site common to many serine

proteases of this family [25]. This suggests that TADG-12 is trafficked to the cell surface where the ligand binding domains are capable of interacting with extracellular molecules and the protease domain is potentially activated. TADG-12 also contains
5 conserved cysteine residues (amino acids 208 and 243) which in other proteases form a disulfide bond capable of linking the activated protease to the other extracellular domains.

EXAMPLE 12

10 Quantitative PCR Characterization of the Alternative Transcript

The original TADG-12 subclone was identified as highly expressed in the initial redundant-primer PCR experiment. The TADG-12 variant form (TADG-12V) with the insertion of 133 bp was also easily detected in the initial experiment. To identify
15 the frequency of this expression and whether or not the expression level between normal ovary and ovarian tumors was different, a previously authenticated semi-quantitative PCR technique was employed [16]. The PCR analysis co-amplified a product for β -tubulin with either a product specific to TADG-12 or
20 TADG-12V in the presence of a radiolabelled nucleotide. The products were separated by agarose gel electrophoresis and a phosphoimager was used to quantitate the relative abundance of each PCR product. Examples of these PCR amplification products are shown for both TADG-12 and TADG-12V in Figure 6. Normal
25 expression was defined as the mean ratio of TADG-12 (or TADG-12V) to β -tubulin $\pm$ 2SD as examined in normal ovarian samples. For tumor samples, overexpression was defined as $>2SD$ from the normal TADG-12/ β -tubulin or TADG-12V/ β -tubulin ratio. The results are summarized in Table 1 and Table 2. TADG-12 was

found to be overexpressed in 41 of 55 carcinomas examined while the variant form was present at aberrantly high levels in 8 of 22 carcinomas. As determined by the student's t test, these differences were statistically significant ($p < 0.05$).

5

TABLE 1**Frequency of Overexpression of TADG-12 in Ovarian Carcinoma**

Histology Type	TADG-12 (%)
Normal	0/16 (0%)
LMP-Serous	3/6 (50%)
LMP-Mucinous	0/4 (0%)
Serous Carcinoma	23/29 (79%)
Mucinous Carcinoma	7/12 (58%)
Endometrioid Carcinoma	8/8 (100%)
Clear Cell Carcinoma	3/6 (50%)
Benign Tumors	3/4 (75%)

10 Overexpression = more than two standard deviations above the mean for normal ovary

LMP = low malignant potential tumor

TABLE 2**Frequency of Overexpression of TADG-12V in Ovarian Carcinoma**

Histology Type	TADG-12V (%)
Normal	0/10 (0%)
LMP-Serous	0/5 (0%)
LMP-Mucinous	0/3 (0%)
Serous Carcinoma	4/14 (29%)
Mucinous Carcinoma	3/5 (60%)
Endometrioid Carcinoma	1/3 (33%)
Clear Cell Carcinoma	N/D

Overexpression =more than two standard deviations above
 5 the mean for normal ovary; LMP = low malignant potential tumor

EXAMPLE 13**Immunohistochemical Analysis of TADG-12 in Ovarian Tumor Cells**

10 In order to examine the TADG-12 protein, polyclonal
 rabbit anti-sera to a peptide located in the carboxy-terminal
 amino acid sequence was developed. These antibodies were used
 to examine the expression level of the TADG-12 protein and its
 localization within normal ovary and ovarian tumor cells by
 15 immuno-localization. No staining was observed in normal ovarian
 tissues (Figure 7A) while significant staining was observed in 22
 of 29 tumors studied. Representative tumor samples are shown in
 Figures 7B and 7C. It should be noted that TADG-12 is found in a
 diffuse pattern throughout the cytoplasm indicative of a protein in
 20 a trafficking pathway. TADG-12 is also found at the cell surface in
 these tumor samples as expected. It should be noted that the

antibody developed and used for immunohistochemical analysis would not detect the TADG-12V truncated protein.

The results of the immunohistochemical staining are summarized in Table 3. 22 of 29 ovarian tumors showed positive staining of TADG-12, whereas normal ovarian surface epithelium showed no expression of the TADG-12 antigen. 8 of 10 serous adenocarcinomas, 8 of 8 mucinous adenocarcinomas, 1 of 2 clear cell carcinomas, and 4 of 6 endometroid carcinomas showed positive staining.

10

TABLE 3

Case	Stage	Histology	Grade	LN*	TADG12	Prognosis
1		Normal ovary			0 -	
2		Normal ovary			0 -	
3		Normal ovary			0 -	
4		Mucinous B		ND	0 -	Alive
5		Mucinous B		ND	1+	Alive
6	1 a	Serous LMP	G1	ND	1+	Alive
7	1 a	Mucinous LMP	G1	ND	1+	Alive
8	1 a	Mucinous CA	G1	ND	1+	Alive
9	1 a	Mucinous CA	G2	ND	1+	Alive
10	1 a	Endometrioid CA	G1	ND	0 -	Alive
11	1 c	Serous CA	G1	N	1+	Alive
12	1 c	Mucinous CA	G1	N	1+	Alive
13	1 c	Mucinous CA	G1	N	2+	Alive
14	1 c	Clear cell CA	G2	N	0 -	Alive
15	1 c	Clear cell CA	G2	N	0 -	Alive
16	2 c	Serous CA	G3	N	2+	Alive
17	3 a	Mucinous CA	G2	N	2+	Alive

18	3 b	Serous CA	G1	ND	1+	Alive
19	3 c	Serous CA	G1	N	0 -	Dead
20	3 c	Serous CA	G3	P	1+	Alive
21	3 c	Serous CA	G2	P	2+	Alive
22	3 c	Serous CA	G1	P	2+	Unknown
23	3 c	Serous CA	G3	ND	2+	Alive
24	3 c	Serous CA	G2	N	0 -	Dead
25	3 c	Mucinous CA	G1	P	2+	Dead
26	3 c	Mucinous CA	G2	ND	1+	Unknown
27	3 c	Mucinous CA	G2	N	1+	Alive
28	3 c	Endometrioid CA	G1	P	1+	Dead
29	3 c	Endometrioid CA	G2	N	0 -	Alive
30	3 c	Endometrioid CA	G2	P	1+	Dead
31	3 c	Endometrioid CA	G3	P	1+	Alive
32	3 c	Clear Cell CA	G3	P	2+	Dead

LN*= Lymph Node: B = Benign; N = Negative; P = Positive;

ND = Not Done

5

EXAMPLE 14

Peptide Ranking

For vaccine or immune stimulation, individual 9-mers to 11-mers of the TADG-12 protein were examined to rank the binding of individual peptides to the top 8 haplotypes in the general population [Parker et al., (1994)], using a computer program made available to the public on the internet by the National Institutes of Health (U.S. government). Table 4 shows the peptide ranking based upon the predicted half-life of each peptide's binding to a particular HLA allele. A larger half-life indicates a

stronger association with that peptide and the particular HLA molecule. The TADG-12 peptides that strongly bind to an HLA allele are putative immunogens, and are used to inoculate an individual against TADG-12.

5

TABLE 4

<u>TADG-12 peptide ranking</u>					
<u>HLA Type</u>		<u>Predicted</u>		<u>SEQ</u>	
<u>& Ranking</u>	<u>Start</u>	<u>Peptide</u>	<u>Dissociation_{1/2}</u>	<u>ID No.</u>	
10	HLA A0201				
	1	40	ILSLLPFEV	685.783	35
	2	144	AQLGFPSYV	545.316	36
	3	225	LLSQWPWQA	63.342	37
	4	252	WIITAAHCV	43.992	38
15	5	356	VLNHAAVPL	36.316	39
	6	176	LLPDDKVT	34.627	40
	7	13	FSFRSLFGL	31.661	41
	8	151	YVSSDNLRV	27.995	42
	9	436	RVTSFLDWI	21.502	43
20	10	234	SLQFQGYHL	21.362	44
	11	181	KVTALHHSV	21.300	45
	12	183	TALHHSVYV	19.658	46
	13	411	RLWKLVGAT	18.494	47
	14	60	LILALAIGL	18.476	48
25	15	227	SQWPWQASL	17.977	49
	16	301	RLGNDIALM	11.426	50
	17	307	ALMKLAGPL	10.275	51
	18	262	DLYLPKSWT	9.837	52
	19	416	LVGATSFGI	9.001	53
30	20	54	SLGIIALIL	8.759	54

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HLA A0205					
5	1	218	IVGGNMSLL	47.600	55
	2	60	LILALAIGL	35.700	48
	3	35	AVAAQILSL	28.000	56
	4	307	ALMKLAGPL	21.000	51
	5	271	IQVGLVSL	19.040	57
10	6	397	CQGDSGGPL	16.800	58
	7	227	SQWPWQASL	16.800	49
	8	270	TIQVGLVSL	14.000	59
	9	56	GIIALILAL	14.000	60
	10	110	RVGGQNAVL	14.000	61
15	11	181	KVTALHHSV	12.000	45
	12	151	YVSSDNLRV	12.000	42
	13	356	VLNHAAPVPL	11.900	39
	14	144	AQLGFPSYV	9.600	36
	15	13	FSFRSLFGL	7.560	41
20	16	54	SLGIIALIL	7.000	54
	17	234	SLQFQGYHL	7.000	44
	18	217	RIVGGNMSL	7.000	62
	19	411	RLWKLVGAT	6.000	47
	20	252	WIITAAHCV	6.000	38
HLA A1					
25	1	130	CSDDWKGHY	37.500	63
	2	8	AVEAPFSFR	9.000	64
	3	328	NSEENFPDG	2.700	65
	4	3	ENDPPAVEA	2.500	66
	5	98	DCKDGEDEY	2.500	67
	6	346	ATEDGGDAS	2.250	68
	7	360	AAVPLISNK	2.000	69

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	8	153	SSDNLRVSS	1.500	70
	9	182	VTALHHSVY	1.250	71
	10	143	CAQLGFPSY	1.000	72
	11	259	CVYDLYLPK	1.000	73
5	12	369	ICNHRDVYG	1.000	74
	13	278	LLDNPAPSH	1.000	75
	14	426	CAEVNKPGV	1.000	76
	15	32	DADAVAAQI	1.000	77
	16	406	VCQERRLWK	1.000	78
10	17	329	SEENFPDGK	0.900	79
	18	303	GNDIALMKL	0.625	80
	19	127	KTMCSDDWK	0.500	81
	20	440	FLDWIHEQM	0.500	82
HLA A24					
15	1	433	VYTRVTSFL	280.000	83
	2	263	LYLPKSWTI	90.000	84
	3	169	EFVSIHLL	42.000	85
	4	217	RIVGGNMSL	12.000	62
	5	296	KYKPKRLGN	12.000	86
20	6	16	RSLFGLDDL	12.000	87
	7	267	KSWTIQVGL	11.200	88
	8	81	RSSFKCIEL	8.800	89
	9	375	VYGGIISPS	8.000	90
	10	110	RVGGQNAVL	8.000	91
25	11	189	VYVREGCAS	7.500	92
	12	60	LILALAIGL	7.200	48
	13	165	QFREEFVSI	7.200	93
	14	271	IQVGLVSL	7.200	57
	15	56	GIIALILAL	7.200	60

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	16	10	EAPFSFRSL	7.200	94
	17	307	ALMKLAGPL	7.200	51
	18	407	CQERRLWKL	6.600	95
	19	356	VLNHAAPVPL	6.000	39
5	20	381	SPSMLCAGY	6.000	96
HLA B7					
	1	375	VYGGIISPS	200.000	97
	2	381	SPSMLCAGY	80.000	98
	3	362	VPLISNKIC	80.000	99
10	4	35	AVAAQILSL	60.000	56
	5	373	RDVYGGIIS	40.000	100
	6	307	ALMKLAGPL	36.000	51
	7	283	APSHLVEKI	24.000	101
	8	177	LPDDKVTAL	24.000	102
15	9	47	EVFSQSSSL	20.000	103
	10	110	RVGGQNAVL	20.000	91
	11	218	IVGGNMSLL	20.000	55
	12	36	VAAQILSLL	12.000	104
	13	255	TAAHCVYDL	12.000	105
20	14	10	EAPFSFRSL	12.000	94
	15	138	YANVACAQL	12.000	106
	16	195	CASGHVVTL	12.000	107
	17	215	SSRIVGGNM	10.00	108
	18	298	KPKRLGNDI	8.000	109
25	19	313	GPLTFNEMI	8.000	110
	20	108	CVRVGGQNA	5.000	111
HLA B8					
	1	294	HSKYKPKRL	80.000	112
	2	373	RDVYGGIIS	16.000	100

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	3	177	LPDDKVTAL	4.800	102
	4	265	LPKSWTIQV	2.400	113
	5	88	ELITRCDGV	2.400	114
	6	298	KPKRLGNDI	2.000	109
5	7	81	RSSFVKCIEL	2.000	89
	8	375	VYGGIISPS	2.000	97
	9	79	RCRSSFKCI	2.000	115
	10	10	EAPFSFRSL	1.600	94
	11	215	SSRIVGGNM	1.000	108
10	12	36	VAAQILSLL	0.800	104
	13	255	TAAHCVYDL	0.800	116
	14	381	SPSMLCAGY	0.800	98
	15	195	CASGHVVTL	0.800	107
	16	362	VPLISNKIC	0.800	99
15	17	138	YANVACAQL	0.800	106
	18	207	ACGHRRGYS	0.400	117
	19	154	SDNLRVSSL	0.400	118
	20	47	EVFSQSSSL	0.400	103
HLA B2702					
20	1	300	KRLGNDIAL	180.000	119
	2	435	TRVTSFLDW	100.000	120
	3	376	YGGIISPSM	100.000	121
	4	410	RRLWKLVGA	60.000	122
	5	210	HRRGYSSRI	60.000	123
25	6	227	SQWPWQASL	30.000	49
	7	109	VRVGGQNAV	20.000	124
	8	191	VREGCASGH	20.000	125
	9	78	YRCRSSFKC	20.000	126
	10	113	GQNAVLQVF	20.000	127

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	1 1	9 1	TRCDGVSDC	20.000	1 2 8
	1 2	3 8	AQILSLLPF	20.000	1 2 9
	1 3	2 1 1	RRGYSSRIV	18.000	1 3 0
	1 4	2 1 6	SRIVGGNMS	10.000	1 3 1
5	1 5	1 1 8	LQVFTAASW	10.000	1 3 2
	1 6	3 7 0	CNHRDVYGG	10.000	1 3 3
	1 7	3 9 3	GVDSCQGDS	10.000	1 3 4
	1 8	2 3 5	LQFQGYHLC	10.000	1 3 5
	1 9	2 7 1	IQVGLVSL	6.000	5 7
10	2 0	4 0 8	CQERRLWKL	6.000	9 5
HLA B4403					
	1	4 2 7	AEVNKPGVY	90.000	1 3 6
	2	1 6 2	LEGQFREEF	40.000	1 3 7
	3	9	VEAPFSFRS	24.000	1 3 8
15	4	3 1 8	NEMIQPVCL	12.000	1 3 9
	5	2 5 6	AAHCVYDLY	9.000	1 4 0
	6	9 8	DCKDGEDEY	9.000	6 7
	7	4 6	FEVFSQSSS	8.000	1 4 1
	8	3 8	AQILSLLPF	7.500	1 2 9
20	9	6 4	LAIGLGIHF	7.500	1 4 2
	1 0	1 9 2	REGCASGHV	6.000	1 4 3
	1 1	3 3 0	EENFPDGKV	6.000	1 4 4
	1 2	1 8 2	VTALHHSVY	6.000	1 4 5
	1 3	4 0 8	QERRLWKL	6.000	1 4 6
25	1 4	2 0 6	TACGHRRGY	4.500	1 4 7
	1 5	5	DPPAVEAPF	4.500	1 4 8
	1 6	2 6 1	YDLYLPKSW	4.500	1 4 9
	1 7	3 3	ADAVAAQIL	4.500	1 5 0
	1 8	1 6 8	EEFVSIDHL	4.000	1 5 1

19	304	NDIALMKLA	3.750	152
20	104	DEYRCVRVG	3.600	153

5 Conclusion

In this study, a serine protease was identified by means of a PCR based strategy. By Northern blot, the largest transcript for this gene is approximately 2.4 kb, and it is found to be expressed at high levels in ovarian tumors while found at minimal levels in all other tissues examined. The full-length cDNA encoding a novel multi-domain, cell-surface serine protease was cloned, named TADG-12. The 454 amino acid protein contains a cytoplasmic domain, a type II transmembrane domain, an LDLR-A domain, an SRCR domain and a serine protease domain. Using a semi-quantitative PCR analysis, it was shown that TADG-12 is overexpressed in a majority of tumors studied. Immunohistochemical staining corroborates that in some cases this protein is localized to the cell-surface of tumor cells and this suggests that TADG-12 has some extracellular proteolytic functions. Interestingly, TADG-12 also has a variant splicing form that is present in 35% of the tumors studied. This variant mRNA would lead to a truncated protein that may provide a unique peptide sequence on the surface of tumor cells.

This protein contains two extracellular domains which might confer unusual properties to this multidomain molecule. Although the precise role of LDLR-A function with regard to proteases remains unclear, this domain certainly has the capacity to bind calcium and other positively charged ligands [21,22]. This may play an important role in the regulation of the protease or

subsequent internalization of the molecule. The SRCR domain was originally identified within the macrophage scavenger receptor and functionally described to bind lipoproteins. Not only are SRCR domains capable of binding lipoproteins, but they may also bind to molecules as diverse as polynucleotides [23]. More recent studies have identified members of this domain family in proteins with functions that vary from proteases to cell adhesion molecules involved in maturation of the immune system [24]. In addition, TADG-12, like TMPRSS2 has only four of six cysteine residues conserved within its SRCR domain. This difference may allow for different structural features of these domains that confer unusual ligand binding properties. At this time, only the function of the CD6 encoded SRCR is well documented. In the case of CD6, the SRCR domain binds to the cell adhesion molecule ALCAM [23]. This mediation of cell adhesion is a useful starting point for future research on newly identified SRCR domains, however, the possibility of multiple functions for this domain can not be overlooked. SRCR domains are certainly capable of cell adhesion type interactions, but their capacity to bind other types of ligands should be considered.

At this time, the precise role of TADG-12 remains unclear. Substrates have not been identified for the protease domain, nor have ligands been identified for the extracellular LDLR-A and SRCR domains. Figure 8 presents a working model of TADG-12 with the information disclosed in the present invention. Two transcripts are produced which lead to the production of either TADG-12 or the truncated TADG-12V proteins. Either of these proteins is potentially targeted to the cell surface. TADG-12 is capable of becoming an activated serine protease while TADG-

12V is a truncated protein product that if at the cell surface may represent a tumor specific epitope.

The problem with treatment of ovarian cancer today remains the inability to diagnose the disease at an early stage.

5 Identifying genes that are expressed early in the disease process such as proteases that are essential for tumor cell growth [26] is an important step toward improving treatment. With this knowledge, it may be possible to design assays to detect the highly expressed genes such as the TADG-12 protease described

10 here or previously described proteases to diagnose these cancers at an earlier stage. Panels of markers may also provide prognostic information and could lead to therapeutic strategies for individual patients. Alternatively, inhibition of enzymes such as proteases may be an effective means for slowing progression of ovarian

15 cancer and improving the quality of patient life. Other features of TADG-12 and TADG-12V must be considered important to future research too. The extracellular ligand binding domains are natural targets for drug delivery systems. The aberrant peptide associated with the TADG-12V protein may provide an excellent

20 target drug delivery or for immune stimulation.

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Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains.

5

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

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15

SEQUENCE LISTING

<110> The Board of Trustees of the University of Arkansas

<120> Transmembrane Serine Protease Overexpressed
in Ovarian Carcinoma and Uses Thereof

<130> PAT 49873W-1

<140> PCT/US00/05612

<141> 2000-03-02

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<221> CDS

<223> entire cDNA sequence of TADG-12 gene

<400> 1

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<210> 2
<211> 454
<212> PRT
<213> Homo sapiens

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<220>
<223> complete amino acid sequence of TADG-12
      protein

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<400> 2

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Pro Asp Ala Asp Ala Val Ala Ala Gln Ile Leu Ser Leu Leu Pro
      35              40              45
Phe Glu Val Phe Ser Gln Ser Ser Ser Leu Gly Ile Ile Ala Leu
      50              55              60
Ile Leu Ala Leu Ala Ile Gly Leu Gly Ile His Phe Asp Cys Ser
      65              70              75
Gly Lys Tyr Arg Cys Arg Ser Ser Phe Lys Cys Ile Glu Leu Ile
      80              85              90
Thr Arg Cys Asp Gly Val Ser Asp Cys Lys Asp Gly Glu Asp Glu
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Tyr Arg Cys Val Arg Val Gly Gly Gln Asn Ala Val Leu Gln Val
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Phe Thr Ala Ala Ser Trp Lys Thr Met Cys Ser Asp Asp Trp Lys
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Gly His Tyr Ala Asn Val Ala Cys Ala Gln Leu Gly Phe Pro Ser	140	145	150
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Phe Arg Glu Glu Phe Val Ser Ile Asp His Leu Leu Pro Asp Asp	170	175	180
Lys Val Thr Ala Leu His His Ser Val Tyr Val Arg Glu Gly Cys	185	190	195
Ala Ser Gly His Val Val Thr Leu Gln Cys Thr Ala Cys Gly His	200	205	210
Arg Arg Gly Tyr Ser Ser Arg Ile Val Gly Gly Asn Met Ser Leu	215	220	225
Leu Ser Gln Trp Pro Trp Gln Ala Ser Leu Gln Phe Gln Gly Tyr	230	235	240
His Leu Cys Gly Gly Ser Val Ile Thr Pro Leu Trp Ile Ile Thr	245	250	255
Ala Ala His Cys Val Tyr Asp Leu Tyr Leu Pro Lys Ser Trp Thr	260	265	270
Ile Gln Val Gly Leu Val Ser Leu Leu Asp Asn Pro Ala Pro Ser	275	280	285
His Leu Val Glu Lys Ile Val Tyr His Ser Lys Tyr Lys Pro Lys	290	295	300
Arg Leu Gly Asn Asp Ile Ala Leu Met Lys Leu Ala Gly Pro Leu	305	310	315
Thr Phe Asn Glu Met Ile Gln Pro Val Cys Leu Pro Asn Ser Glu	320	325	330
Glu Asn Phe Pro Asp Gly Lys Val Cys Trp Thr Ser Gly Trp Gly	335	340	345
Ala Thr Glu Asp Gly Gly Asp Ala Ser Pro Val Leu Asn His Ala	350	355	360
Ala Val Pro Leu Ile Ser Asn Lys Ile Cys Asn His Arg Asp Val	365	370	375
Tyr Gly Gly Ile Ile Ser Pro Ser Met Leu Cys Ala Gly Tyr Leu	380	385	390
Thr Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu	395	400	405
Val Cys Gln Glu Arg Arg Leu Trp Lys Leu Val Gly Ala Thr Ser	410	415	420
Phe Gly Ile Gly Cys Ala Glu Val Asn Lys Pro Gly Val Tyr Thr	425	430	435
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Asp Leu Lys Thr			

<210> 3
 <211> 2544
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <223> entire cDNA sequence of TADG-12 variant gene

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<210> 4
 <211> 294
 <212> PRT
 <213> Homo sapiens

<220>
 <223> complete amino acid sequence of TADG-12
 variant protein

<400> 4

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Pro	Asp	Ala	Asp	Ala	Val	Ala	Ala	Gln	Ile	Leu	Ser	Leu	Leu	Pro	35	40	45
Phe	Glu	Val	Phe	Ser	Gln	Ser	Ser	Ser	Leu	Gly	Ile	Ile	Ala	Leu	50	55	60
Ile	Leu	Ala	Leu	Ala	Ile	Gly	Leu	Gly	Ile	His	Phe	Asp	Cys	Ser	65	70	75
Gly	Lys	Tyr	Arg	Cys	Arg	Ser	Ser	Phe	Lys	Cys	Ile	Glu	Leu	Ile	80	85	90
Thr	Arg	Cys	Asp	Gly	Val	Ser	Asp	Cys	Lys	Asp	Gly	Glu	Asp	Glu	95	100	105
Tyr	Arg	Cys	Val	Arg	Val	Gly	Gly	Gln	Asn	Ala	Val	Leu	Gln	Val	110	115	120
Phe	Thr	Ala	Ala	Ser	Trp	Lys	Thr	Met	Cys	Ser	Asp	Asp	Trp	Lys	125	130	135
Gly	His	Tyr	Ala	Asn	Val	Ala	Cys	Ala	Gln	Leu	Gly	Phe	Pro	Ser	140	145	150
Tyr	Val	Ser	Ser	Asp	Asn	Leu	Arg	Val	Ser	Ser	Leu	Glu	Gly	Gln	155	160	165
Phe	Arg	Glu	Glu	Phe	Val	Ser	Ile	Asp	His	Leu	Leu	Pro	Asp	Asp	170	175	180
Lys	Val	Thr	Ala	Leu	His	His	Ser	Val	Tyr	Val	Arg	Glu	Gly	Cys	185	190	195
Ala	Ser	Gly	His	Val	Val	Thr	Leu	Gln	Cys	Thr	Ala	Cys	Gly	His	200	205	210
Arg	Arg	Gly	Tyr	Ser	Ser	Arg	Ile	Val	Gly	Gly	Asn	Met	Ser	Leu	215	220	225
Leu	Ser	Gln	Trp	Pro	Trp	Gln	Ala	Ser	Leu	Gln	Phe	Gln	Gly	Tyr	230	235	240
His	Leu	Cys	Gly	Gly	Ser	Val	Ile	Thr	Pro	Leu	Trp	Ile	Ile	Thr	245	250	255
Ala	Ala	His	Cys	Val	Tyr	Glu	Ile	Val	Ala	Pro	Arg	Glu	Arg	Ala	260	265	270
Asp	Arg	Arg	Gly	Arg	Lys	Leu	Leu	Cys	Trp	Arg	Lys	Pro	Thr	Lys	275	280	285
Met	Lys	Gly	Pro	Arg	Pro	Ser	His	Ser							290		

<210> 5
 <211> 174

<212> DNA
 <213> Artificial sequence

<220>

<223> nucleotide sequence of the subclone containing
 the 180 bp band from the PCR product for TADG-12

<400> 5

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cccacttggt	ggagaagatt	gtctaccaca	gcaagtacaa	gccaaagagg	150
ctgggcaacg	acatcgccct	ccta			174

<210> 6
 <211> 58
 <212> PRT
 <213> Artificial sequence

<220>

<223> deduced amino acid sequence of the 180 bp band
 from the PCR product for TADG-12

<400> 6

Trp	Val	Val	Thr	Ala	Ala	His	Cys	Val	Tyr	Asp	Leu	Tyr	Leu	Pro
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Lys	Ser	Trp	Thr	Ile	Gln	Val	Gly	Leu	Val	Ser	Leu	Leu	Asp	Asn
				20					25					30
Pro	Ala	Pro	Ser	His	Leu	Val	Glu	Lys	Ile	Val	Tyr	His	Ser	Lys
				35					40					45
Tyr	Lys	Pro	Lys	Arg	Leu	Gly	Asn	Asp	Ile	Ala	Leu	Leu		
				50					55					

<210> 7
 <211> 328
 <212> DNA
 <213> Artificial sequence

<220>

<223> nucleotide sequence of the subclone containing
 the 300 bp band from the PCR product for
 TADG-12 variant, which contains an additional
 insert of 133 bases

<400> 7

gggtggtgac	ggcggcgcac	tgtgtttatg	agattgtagc	tcctagagaa	50
agggcagaca	gaagaggaag	gaagctcctg	tgctggagga	aaccacaaa	100
aatgaaagga	cctagacctt	cccatagcta	attccagtgg	accatgttat	150
ggcagataca	ggcttgtacc	tccccaagtc	atggaccatc	caggtgggtc	200
tagtttcct	gttggacaat	ccagcccat	cccacttggt	ggagaagatt	250

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 cctaatacact agtgcggccg cctgcagg 328

<210> 8
 <211> 42
 <212> PRT
 <213> Artificial sequence

<220>
 <223> deduced amino acid sequence of the 300 bp band
 from the PCR product for TADG-12 variant, which is
 a truncated form of TADG-12

<400> 8

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

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

<220>
 <221> DOMAIN
 <223> LDLR-A domain of the complement subunit C8
 (CompC8)

<400> 9

Cys	Glu	Gly	Phe	Val	Cys	Ala	Gln	Thr	Gly	Arg	Cys	Val	Asn	Arg
				5					10					15
Arg	Leu	Leu	Cys	Asn	Gly	Asp	Asn	Asp	Cys	Gly	Asp	Gln	Ser	Asp
				20					25					30
Glu	Ala	Asn	Cys											

<210> 10
 <211> 34
 <212> PRT
 <213> Homo sapiens

<220>
 <221> DOMAIN
 <223> LDLR-A domain of the serine protease
 matriptase (Matr)

<400> 10

Cys	Pro	Gly	Gln	Phe	Thr	Cys	Arg	Thr	Gly	Arg	Cys	Ile	Arg	Lys
				5					10					15
Glu	Leu	Arg	Cys	Asp	Gly	Trp	Ala	Asp	Cys	Thr	Asp	His	Ser	Asp
				20					25					30
Glu	Leu	Asn	Cys											

<210> 11

<211> 37

<212> PRT

<213> Homo sapiens

<220>

<221> DOMAIN

<223> LDLR-A domain of the glycoprotein GP300
(Gp300-1)

<400> 11

Cys	Gln	Gln	Gly	Tyr	Phe	Lys	Cys	Gln	Ser	Glu	Gly	Gln	Cys	Ile
				5					10					15
Pro	Ser	Ser	Trp	Val	Cys	Asp	Gln	Asp	Gln	Asp	Cys	Asp	Asp	Gly
				20					25					30
Ser	Asp	Glu	Arg	Gln	Asp	Cys								
				35										

<210> 12

<211> 35

<212> PRT

<213> Homo sapiens

<220>

<221> DOMAIN

<223> LDLR-A domain of the glycoprotein GP300
(Gp300-2)

<400> 12

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				5					10					15
Ser	Glu	Tyr	Arg	Cys	Asp	His	Val	Arg	Asp	Cys	Pro	Asp	Gly	Ala
				20					25					30
Asp	Glu	Asn	Asp	Cys										
				35										

<210> 13

<211> 35

<212> PRT

<213> Homo sapiens

<220>

<221> DOMAIN

<222> 74...108

<223> LDLR-A domain of TADG-12

<400> 13

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Leu	Ile	Thr	Arg	Cys	Asp	Gly	Val	Ser	Asp	Cys	Lys	Asp	Gly	Glu
				20					25					30
Asp	Glu	Tyr	Arg	Cys										
				35										

<210> 14

<211> 36

<212> PRT

<213> Homo sapiens

<220>

<221> DOMAIN

<223> LDLR-A domain of the serine protease TMPRSS2
Tmprss2

<400> 14

Cys	Ser	Asn	Ser	Gly	Ile	Glu	Cys	Asp	Ser	Ser	Gly	Thr	Cys	Ile
				5					10					15
Asn	Pro	Ser	Asn	Trp	Cys	Asp	Gly	Val	Ser	His	Cys	Pro	Gly	Gly
				20					25					30
Glu	Asp	Glu	Asn	Arg	Cys									
				35										

<210> 15

<211> 101

<212> PRT

<213> Bos taurus

<220>

<221> DOMAIN

<223> SRCR domain of bovine enterokinase (BovEntk)

<400> 15

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Ile	Phe	His	Glu	Gly	Gln	Trp	Gly	Thr	Val	Cys	Asp	Asp	Arg	Trp
				20					25					30
Glu	Leu	Arg	Gly	Gly	Leu	Val	Val	Cys	Arg	Ser	Leu	Gly	Tyr	Lys
				35					40					45

Gly	Val	Gln	Ser	Val	His	Lys	Arg	Ala	Tyr	Phe	Gly	Lys	Gly	Thr
				50					55					60
Gly	Pro	Ile	Trp	Leu	Asn	Glu	Val	Phe	Cys	Phe	Gly	Lys	Glu	Ser
				65					70					75
Ser	Ile	Glu	Glu	Cys	Arg	Ile	Arg	Gln	Trp	Gly	Val	Arg	Ala	Cys
				80					85					90
Ser	His	Asp	Glu	Asp	Ala	Gly	Val	Thr	Cys	Thr				
				95					100					

<210> 16
 <211> 101
 <212> PRT
 <213> Homo sapiens

<220>
 <221> DOMAIN
 <223> SRCR domain of human macrophage scavenger
 receptor (MacSR)

<400> 16

Val	Arg	Leu	Val	Gly	Gly	Ser	Gly	Pro	His	Glu	Gly	Arg	Val	Glu
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Ile	Leu	His	Ser	Gly	Gln	Trp	Gly	Thr	Ile	Cys	Asp	Asp	Arg	Trp
				20					25					30
Glu	Val	Arg	Val	Gly	Gln	Val	Val	Cys	Arg	Ser	Leu	Gly	Tyr	Pro
				35					40					45
Gly	Val	Gln	Ala	Val	His	Lys	Ala	Ala	His	Phe	Gly	Gln	Gly	Thr
				50					55					60
Gly	Pro	Ile	Trp	Leu	Asn	Glu	Val	Phe	Cys	Phe	Gly	Arg	Glu	Ser
				65					70					75
Ser	Ile	Glu	Glu	Cys	Lys	Ile	Arg	Gln	Trp	Gly	Thr	Arg	Ala	Cys
				80					85					90
Ser	His	Ser	Glu	Asp	Ala	Gly	Val	Thr	Cys	Thr				
				95					100					

<210> 17
 <211> 98
 <212> PRT
 <213> Homo sapiens

<220>
 <221> DOMAIN
 <222> 109...206
 <223> SRCR domain of TADG-12 (TADG12)

<400> 17

Val	Arg	Val	Gly	Gly	Gln	Asn	Ala	Val	Leu	Gln	Val	Phe	Thr	Ala
				5					10					15
Ala	Ser	Trp	Lys	Thr	Met	Cys	Ser	Asp	Asp	Trp	Lys	Gly	His	Tyr
				20					25					30

Ala	Asn	Val	Ala	Cys	Ala	Gln	Leu	Gly	Phe	Pro	Ser	Tyr	Val	Ser
				35					40					45
Ser	Asp	Asn	Leu	Arg	Val	Ser	Ser	Leu	Glu	Gly	Gln	Phe	Arg	Glu
				50					55					60
Glu	Phe	Val	Ser	Ile	Asp	His	Leu	Leu	Pro	Asp	Asp	Lys	Val	Thr
				65					70					75
Ala	Leu	His	His	Ser	Val	Tyr	Val	Arg	Glu	Gly	Cys	Ala	Ser	Gly
				80					85					90
His	Val	Val	Thr	Leu	Gln	Cys	Thr							
				95										

<210> 18
 <211> 94
 <212> PRT
 <213> Homo sapiens

<220>
 <221> DOMAIN
 <223> SRCR domain of the serine protease TMPRSS2
 (Tmprss2)

<400> 18

Val	Arg	Leu	Tyr	Gly	Pro	Asn	Phe	Ile	Leu	Gln	Met	Tyr	Ser	Ser
				5					10					15
Gln	Arg	Lys	Ser	Trp	His	Pro	Val	Cys	Gln	Asp	Asp	Trp	Asn	Glu
				20					25					30
Asn	Tyr	Gly	Arg	Ala	Ala	Cys	Arg	Asp	Met	Gly	Tyr	Lys	Asn	Asn
				35					40					45
Phe	Tyr	Ser	Ser	Gln	Gly	Ile	Val	Asp	Asp	Ser	Gly	Ser	Thr	Ser
				50					55					60
Phe	Met	Lys	Leu	Asn	Thr	Ser	Ala	Gly	Asn	Val	Asp	Ile	Tyr	Lys
				65					70					75
Lys	Leu	Tyr	His	Ser	Asp	Ala	Cys	Ser	Ser	Lys	Ala	Val	Val	Ser
				80					85					90
Leu	Arg	Cys	Leu											

<210> 19
 <211> 90
 <212> PRT
 <213> Homo sapiens

<220>
 <221> DOMAIN
 <223> SRCR domain of human enterokinase (HumEntk)

<400> 19

Val	Arg	Phe	Phe	Asn	Gly	Thr	Thr	Asn	Asn	Asn	Gly	Leu	Val	Arg
				5				10						15

Phe	Arg	Ile	Gln	Ser	Ile	Trp	His	Thr	Ala	Cys	Ala	Glu	Asn	Trp
				20					25					30
Thr	Thr	Gln	Ile	Ser	Asn	Asp	Val	Cys	Gln	Leu	Leu	Gly	Leu	Gly
				35					40					45
Ser	Gly	Asn	Ser	Ser	Lys	Pro	Ile	Phe	Ser	Thr	Asp	Gly	Gly	Pro
				50					55					60
Phe	Val	Lys	Leu	Asn	Thr	Ala	Pro	Asp	Gly	His	Leu	Ile	Leu	Thr
				65					70					75
Pro	Ser	Gln	Gln	Cys	Leu	Gln	Asp	Ser	Leu	Ile	Arg	Leu	Gln	Cys
				80					85					90

<210> 20
 <211> 149
 <212> PRT
 <213> Homo sapiens

<220>
 <221> DOMAIN
 <223> protease domain of protease M (ProM)

Leu	Trp	Val	Leu	Thr	Ala	Ala	His	Cys	Lys	Lys	Pro	Asn	Leu	Gln
				5					10					15
Val	Phe	Leu	Gly	Lys	His	Asn	Leu	Arg	Gln	Arg	Glu	Ser	Ser	Gln
				20					25					30
Glu	Gln	Ser	Ser	Val	Val	Arg	Ala	Val	Ile	His	Pro	Asp	Tyr	Asp
				35					40					45
Ala	Ala	Ser	His	Asp	Gln	Asp	Ile	Met	Leu	Leu	Arg	Leu	Ala	Arg
				50					55					60
Pro	Ala	Lys	Leu	Ser	Glu	Leu	Ile	Gln	Pro	Leu	Pro	Leu	Glu	Arg
				65					70					75
Asp	Cys	Ser	Ala	Asn	Thr	Thr	Ser	Cys	His	Ile	Leu	Gly	Trp	Gly
				80					85					90
Lys	Thr	Ala	Asp	Gly	Asp	Phe	Pro	Asp	Thr	Ile	Gln	Cys	Ala	Tyr
				95					100					105
Ile	His	Leu	Val	Ser	Arg	Glu	Glu	Cys	Glu	His	Ala	Tyr	Pro	Gly
				110					115					120
Gln	Ile	Thr	Gln	Asn	Met	Leu	Cys	Ala	Gly	Asp	Glu	Lys	Tyr	Gly
				125					130					135
Lys	Asp	Ser	Cys	Gln	Gly	Asp	Ser	Gly	Gly	Pro	Leu	Val	Cys	
				140					145					

<210> 21
 <211> 151
 <212> PRT
 <213> Homo sapiens

<220>
 <221> DOMAIN
 <223> protease domain of trypsinogen I (Try1)

<400> 21

Gln	Trp	Val	Val	Ser	Ala	Gly	His	Cys	Tyr	Lys	Ser	Arg	Ile	Gln
				5					10					15
Val	Arg	Leu	Gly	Glu	His	Asn	Ile	Glu	Val	Leu	Glu	Gly	Asn	Glu
				20					25					30
Gln	Phe	Ile	Asn	Ala	Ala	Lys	Ile	Ile	Arg	His	Pro	Gln	Tyr	Asp
				35					40					45
Arg	Lys	Thr	Leu	Asn	Asn	Asp	Ile	Met	Leu	Ile	Lys	Leu	Ser	Ser
				50					55					60
Arg	Ala	Val	Ile	Asn	Ala	Arg	Val	Ser	Thr	Ile	Ser	Leu	Pro	Thr
				65					70					75
Ala	Pro	Pro	Ala	Thr	Gly	Thr	Lys	Cys	Leu	Ile	Ser	Gly	Trp	Gly
				80					85					90
Asn	Thr	Ala	Ser	Ser	Gly	Ala	Asp	Tyr	Pro	Asp	Glu	Leu	Gln	Cys
				95					100					105
Leu	Asp	Ala	Pro	Val	Leu	Ser	Gln	Ala	Lys	Cys	Glu	Ala	Ser	Tyr
				110					115					120
Pro	Gly	Lys	Ile	Thr	Ser	Asn	Met	Phe	Cys	Val	Gly	Phe	Leu	Glu
				125					130					135
Gly	Gly	Lys	Asp	Ser	Cys	Gln	Gly	Asp	Ser	Gly	Gly	Pro	Val	Val
				140					145					150

Cys

<210> 22
 <211> 158
 <212> PRT
 <213> Homo sapiens

<220>
 <221> DOMAIN
 <223> protease domain of plasma kallikrein (Kal)

<400> 22

Gln	Trp	Val	Leu	Thr	Ala	Ala	His	Cys	Phe	Asp	Gly	Leu	Pro	Leu
				5					10					15
Gln	Asp	Val	Trp	Arg	Ile	Tyr	Ser	Gly	Ile	Leu	Asn	Leu	Ser	Asp
				20					25					30
Ile	Thr	Lys	Asp	Thr	Pro	Phe	Ser	Gln	Ile	Lys	Glu	Ile	Ile	Ile
				35					40					45
His	Gln	Asn	Tyr	Lys	Val	Ser	Glu	Gly	Asn	His	Asp	Ile	Ala	Leu
				50					55					60
Ile	Lys	Leu	Gln	Ala	Pro	Leu	Asn	Tyr	Thr	Glu	Phe	Gln	Lys	Pro
				65					70					75
Ile	Cys	Leu	Pro	Ser	Lys	Gly	Asp	Thr	Ser	Thr	Ile	Tyr	Thr	Asn
				80					85					90
Cys	Trp	Val	Thr	Gly	Trp	Gly	Phe	Ser	Lys	Glu	Lys	Gly	Glu	Ile
				95					100					105
Gln	Asn	Ile	Leu	Gln	Lys	Val	Asn	Ile	Pro	Leu	Val	Thr	Asn	Glu
				110					115					120
Glu	Cys	Gln	Lys	Arg	Tyr	Gln	Asp	Tyr	Lys	Ile	Thr	Gln	Arg	Met
				125					130					135
Val	Cys	Ala	Gly	Tyr	Lys	Glu	Gly	Gly	Lys	Asp	Ala	Cys	Lys	Gly
				140					145					150

Asp Ser Gly Gly Pro Leu Val Cys
155

<210> 23
<211> 157
<212> PRT
<213> Homo sapiens

<220>
<221> DOMAIN
<223> protease domain of TADG-12 (TADG12)

<400> 23

Leu	Trp	Ile	Ile	Thr	Ala	Ala	His	Cys	Val	Tyr	Asp	Leu	Tyr	Leu
				5					10					15
Pro	Lys	Ser	Trp	Thr	Ile	Gln	Val	Gly	Leu	Val	Ser	Leu	Leu	Asp
				20					25					30
Asn	Pro	Ala	Pro	Ser	His	Leu	Val	Glu	Lys	Ile	Val	Tyr	His	Ser
				35					40					45
Lys	Tyr	Lys	Pro	Lys	Arg	Leu	Gly	Asn	Asp	Ile	Ala	Leu	Met	Lys
				50					55					60
Leu	Ala	Gly	Pro	Leu	Thr	Phe	Asn	Glu	Met	Ile	Gln	Pro	Val	Cys
				65					70					75
Leu	Pro	Asn	Ser	Glu	Glu	Asn	Phe	Pro	Asp	Gly	Lys	Val	Cys	Trp
				80					85					90
Thr	Ser	Gly	Trp	Gly	Ala	Thr	Glu	Asp	Gly	Gly	Asp	Ala	Ser	Pro
				95					100					105
Val	Leu	Asn	His	Ala	Ala	Val	Pro	Leu	Ile	Ser	Asn	Lys	Ile	Cys
				110					115					120
Asn	His	Arg	Asp	Val	Tyr	Gly	Gly	Ile	Ile	Ser	Pro	Ser	Met	Leu
				125					130					135
Cys	Ala	Gly	Tyr	Leu	Thr	Gly	Gly	Val	Asp	Ser	Cys	Gln	Gly	Asp
				140					145					150
Ser	Gly	Gly	Pro	Leu	Val	Cys								
				155										

<210> 24
<211> 159
<212> PRT
<213> Homo sapiens

<220>
<221> DOMAIN
<223> protease domain of TMPRSS2 (Tmprss2)

<400> 24

Glu	Trp	Ile	Val	Thr	Ala	Ala	His	Cys	Val	Glu	Lys	Pro	Leu	Asn
				5					10					15
Asn	Pro	Trp	His	Trp	Thr	Ala	Phe	Ala	Gly	Ile	Leu	Arg	Gln	Ser
				20					25					30

Phe	Met	Phe	Tyr	Gly	Ala	Gly	Tyr	Gln	Val	Gln	Lys	Val	Ile	Ser	
				35					40					45	
His	Pro	Asn	Tyr	Asp	Ser	Lys	Thr	Lys	Asn	Asn	Asp	Ile	Ala	Leu	
				50					55					60	
Met	Lys	Leu	Gln	Lys	Pro	Leu	Thr	Phe	Asn	Asp	Leu	Val	Lys	Pro	
				65					70					75	
Val	Cys	Leu	Pro	Asn	Pro	Gly	Met	Met	Leu	Gln	Pro	Glu	Gln	Leu	
				80					85					90	
Cys	Trp	Ile	Ser	Gly	Trp	Gly	Ala	Thr	Glu	Glu	Lys	Gly	Lys	Thr	
				95					100					105	
Ser	Glu	Val	Leu	Asn	Ala	Ala	Lys	Val	Leu	Leu	Ile	Glu	Thr	Gln	
				110					115					120	
Arg	Cys	Asn	Ser	Arg	Tyr	Val	Tyr	Asp	Asn	Leu	Ile	Thr	Pro	Ala	
				125					130					135	
Met	Ile	Cys	Ala	Gly	Phe	Leu	Gln	Gly	Asn	Val	Asp	Ser	Cys	Gln	
				140					145					150	
Gly	Asp	Ser	Gly	Gly	Pro	Leu	Val	Thr							
				155											

<210> 25

<211> 164

<212> PRT

<213> Homo sapiens

<220>

<221> DOMAIN

<223> protease domain of Hepsin (Heps)

<400> 25

Asp	Trp	Val	Leu	Thr	Ala	Ala	His	Cys	Phe	Pro	Glu	Arg	Asn	Arg	
				5					10					15	
Val	Leu	Ser	Arg	Trp	Arg	Val	Phe	Ala	Gly	Ala	Val	Ala	Gln	Ala	
				20					25					30	
Ser	Pro	His	Gly	Leu	Gln	Leu	Gly	Val	Gln	Ala	Val	Val	Tyr	His	
				35					40					45	
Gly	Gly	Tyr	Leu	Pro	Phe	Arg	Asp	Pro	Asn	Ser	Glu	Glu	Asn	Ser	
				50					55					60	
Asn	Asp	Ile	Ala	Leu	Val	His	Leu	Ser	Ser	Pro	Leu	Pro	Leu	Thr	
				65					70					75	
Glu	Tyr	Ile	Gln	Pro	Val	Cys	Leu	Pro	Ala	Ala	Gly	Gln	Ala	Leu	
				80					85					90	
Val	Asp	Gly	Lys	Ile	Cys	Thr	Val	Thr	Gly	Trp	Gly	Asn	Thr	Gln	
				95					100					105	
Tyr	Tyr	Gly	Gln	Gln	Ala	Gly	Val	Leu	Gln	Glu	Ala	Arg	Val	Pro	
				110					115					120	
Ile	Ile	Ser	Asn	Asp	Val	Cys	Asn	Gly	Ala	Asp	Phe	Tyr	Gly	Asn	
				125					130					135	
Gln	Ile	Lys	Pro	Lys	Met	Phe	Cys	Ala	Gly	Tyr	Pro	Glu	Gly	Gly	
				140					145					150	
Ile	Asp	Ala	Cys	Gln	Gly	Asp	Ser	Gly	Gly	Pro	Phe	Val	Cys		
				155					160						

<210> 26
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <221> primer_bind
 <222> 6, 9, 12, 15, 18
 <223> forward redundant primer for the consensus
 sequences of amino acids surrounding the catalytic
 triad for serine proteases, n = inosine

<400> 26

tgggtngtna cngcngcnca ytg

23

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

<220>
 <221> primer_bind
 <222> 3, 6, 9, 12, 15, 18
 <223> reverse redundant primer for the consensus
 sequences of amino acids surrounding the catalytic
 triad for serine proteases, n = inosine

<400> 27

arnarngcna tntcnttncc

20

<210> 28
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <221> primer_bind
 <223> forward oligonucleotide primer for TADG-12
 used for quantitative PCR

<400> 28

gaaacatgtc cttgctctcg

20

<210> 29
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <221> primer_bind
 <223> reverse oligonucleotide primer for TADG-12
 used for quantitative PCR

<400> 29

actaacttcc acagcctcct

20

<210> 30
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <221> primer_bind
 <223> forward oligonucleotide primer for TADG-12
 variant (TADG-12V) used for quantitative PCR

<400> 30

tccaggtggg tctagtttcc

20

<210> 31
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <221> primer_bind
 <223> reverse oligonucleotide primer for TADG-12
 variant (TADG-12V) used for quantitative PCR

<400> 31

ctcttttggt tgtacttgct

20

<210> 32
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <221> primer_bind
 <223> forward oligonucleotide primer for (-tubulin
 used as an internal control for quantitative PCR

<400> 32

cgcataacg tgtactacaa

20

<210> 33
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <221> primer_bind
 <223> reverse oligonucleotide primer for (-tubulin
 used as an internal control for quantitative PCR

<400> 33

tacgagctgg tggactgaga

20

<210> 34
 <211> 12
 <212> PRT
 <213> Artificial sequence

<220>
 <223> a poly-lysine linked multiple antigen peptide
 derived from the TADG-12 carboxy-terminal protein
 sequence, present in full length TADG-12, but not
 in TADG-12V

<400> 34

Trp Ile His Glu Gln Met Glu Arg Asp Leu Lys Thr
 5 10

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

<220>
 <222> 40...48
 <223> TADG-12 peptide

<400> 35

Ile Leu Ser Leu Leu Pro Phe Glu Val
 5

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

<220>
 <222> 144...152
 <223> TADG-12 peptide

<400> 36

Ala Gln Leu Gly Phe Pro Ser Tyr Val
 5

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

<220>
 <222> 225...233
 <223> TADG-12 peptide

<400> 37

Leu Leu Ser Gln Trp Pro Trp Gln Ala
 5

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

<220>
 <222> 252...260
 <223> TADG-12 peptide

<400> 38

Trp Ile Ile Thr Ala Ala His Cys Val
 5

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

<220>
 <222> 356...364
 <223> TADG-12 peptide

<400> 39

Val Leu Asn His Ala Ala Val Pro Leu
 5

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

<220>
 <222> 176...184
 <223> TADG-12 peptide

<400> 40

Leu Leu Pro Asp Asp Lys Val Thr Ala
 5

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

<220>
 <222> 13...21
 <223> TADG-12 peptide

<400> 41

Phe Ser Phe Arg Ser Leu Phe Gly Leu
 5

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

<220>
 <222> 151...159
 <223> TADG-12 peptide

<400> 42

Tyr Val Ser Ser Asp Asn Leu Arg Val
 5

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

<220>

<222> 436...444
 <223> TADG-12 peptide

<400> 43

Arg Val Thr Ser Phe Leu Asp Trp Ile
 5

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

<220>
 <222> 234...242
 <223> TADG-12 peptide

<400> 44

Ser Leu Gln Phe Gln Gly Tyr His Leu
 5

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

<220>
 <222> 181...189
 <223> TADG-12 peptide

<400> 45

Lys Val Thr Ala Leu His His Ser Val
 5

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

<220>
 <222> 183...191
 <223> TADG-12 peptide

<400> 46

Thr Ala Leu His His Ser Val Tyr Val
 5

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

<220>
 <222> 411...419
 <223> TADG-12 peptide

<400> 47

Arg Leu Trp Lys Leu Val Gly Ala Thr
 5

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

<220>
 <222> 60...68
 <223> TADG-12 peptide

<400> 48

Leu Ile Leu Ala Leu Ala Ile Gly Leu
 5

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

<220>
 <222> 227...235
 <223> TADG-12 peptide

<400> 49

Ser Gln Trp Pro Trp Gln Ala Ser Leu
 5

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

<220>
 <222> 301...309

<223> TADG-12 peptide

<400> 50

Arg Leu Gly Asn Asp Ile Ala Leu Met
5

<210> 51

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<222> 307...315

<223> TADG-12 peptide

<400> 51

Ala Leu Met Lys Leu Ala Gly Pro Leu
5

<210> 52

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<222> 262...270

<223> TADG-12 peptide

<400> 52

Asp Leu Tyr Leu Pro Lys Ser Trp Thr
5

<210> 53

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<222> 416...424

<223> TADG-12 peptide

<400> 53

Leu Val Gly Ala Thr Ser Phe Gly Ile
5

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

<220>
 <222> 54...62
 <223> TADG-12 peptide

<400> 54

Ser Leu Gly Ile Ile Ala Leu Ile Leu
 5

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

<220>
 <222> 218...226
 <223> TADG-12 peptide

<400> 55

Ile Val Gly Gly Asn Met Ser Leu Leu
 5

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

<220>
 <222> 35...43
 <223> TADG-12 peptide

<400> 56

Ala Val Ala Ala Gln Ile Leu Ser Leu
 5

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

<220>
 <222> 271...279
 <223> TADG-12 peptide

<400> 57

Ile Gln Val Gly Leu Val Ser Leu Leu
5

<210> 58

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<222> 397...405

<223> TADG-12 peptide

<400> 58

Cys Gln Gly Asp Ser Gly Gly Pro Leu
5

<210> 59

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<222> 270...278

<223> TADG-12 peptide

<400> 59

Thr Ile Gln Val Gly Leu Val Ser Leu
5

<210> 60

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<222> 56...64

<223> TADG-12 peptide

<400> 60

Gly Ile Ile Ala Leu Ile Leu Ala Leu
5

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

<220>
 <222> 110...118
 <223> TADG-12 peptide

<400> 61

Arg Val Gly Gly Gln Asn Ala Val Leu
 5

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

<220>
 <222> 217...225
 <223> TADG-12 peptide

<400> 62

Arg Ile Val Gly Gly Asn Met Ser Leu
 5

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

<220>
 <222> 130...138
 <223> TADG-12 peptide

<400> 63

Cys Ser Asp Asp Trp Lys Gly His Tyr
 5

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

<220>
 <222> 8...16
 <223> TADG-12 peptide

<400> 64

Ala Val Glu Ala Pro Phe Ser Phe Arg
5

<210> 65

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<222> 328...336

<223> TADG-12 peptide

<400> 65

Asn Ser Glu Glu Asn Phe Pro Asp Gly
5

<210> 66

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<222> 3...11

<223> TADG-12 peptide

<400> 66

Glu Asn Asp Pro Pro Ala Val Glu Ala
5

<210> 67

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<222> 98...106

<223> TADG-12 peptide

<400> 67

Asp Cys Lys Asp Gly Glu Asp Glu Tyr
5

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

<220>
 <222> 346...354
 <223> TADG-12 peptide

<400> 68

Ala Thr Glu Asp Gly Gly Asp Ala Ser
 5

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

<220>
 <222> 360...368
 <223> TADG-12 peptide

<400> 69

Ala Ala Val Pro Leu Ile Ser Asn Lys
 5

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

<220>
 <222> 153...161
 <223> TADG-12 peptide

<400> 70

Ser Ser Asp Asn Leu Arg Val Ser Ser
 5

<210> 71
 <211> 9
 <212> PRT
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Cys Ala Ser Gly His Val Val Thr Leu
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Gly Pro Leu Thr Phe Asn Glu Met Ile
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Ala Glu Val Asn Lys Pro Gly Val Tyr
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Ala Ala His Cys Val Tyr Asp Leu Tyr
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<210> 143

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Glu Glu Asn Phe Pro Asp Gly Lys Val
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Val Thr Ala Leu His His Ser Val Tyr
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Thr Ala Cys Gly His Arg Arg Gly Tyr
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Asp Pro Pro Ala Val Glu Ala Pro Phe
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Tyr Asp Leu Tyr Leu Pro Lys Ser Trp
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Ala Asp Ala Val Ala Ala Gln Ile Leu
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Glu Glu Phe Val Ser Ile Asp His Leu
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Asp Glu Tyr Arg Cys Val Arg Val Gly
5

CLAIMS:

1. A DNA fragment encoding Tumor Antigen Derived Gene-12 protein (TADG-12) of amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4 that is:
 - (a) an isolated DNA fragment that encodes a TADG-12 protein of amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4;
 - (b) an isolated DNA fragment which, when hybridized for 60 minutes at 68°C in the presence of a hybridization buffer comprising sodium dodecyl sulfate, hybridizes to the complement of isolated DNA of (a) above and which encodes a TADG-12 protein of amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4; or
 - (c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a Tumor Antigen Derived Gene-12 protein (TADG-12) of amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4.
2. The DNA fragment of claim 1, wherein said DNA fragment consists of a sequence of SEQ ID NO:1 or SEQ ID NO:3.
3. A vector comprising the DNA fragment according to claim 1 or 2, and regulatory elements necessary for expression of the DNA in a cell.
4. The vector according to claim 3, wherein said DNA encodes a TADG-12 protein consisting of the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4.
5. A host cell transfected with the vector of claim 4, said vector expressing a TADG-12 protein of amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4.
6. The host cell of claim 5, wherein said cell is a bacterial cell, a mammalian cell, a plant cell or an insect cell.
7. The host cell of claim 6, wherein said bacterial cell is *E. coli*.

8. An antisense oligonucleotide directed against the DNA fragment according to claim 1.
9. An isolated and purified Tumor Antigen Derived Gene-12 protein (TADG-12) of amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4, encoded for by a DNA molecule that is:
 - (a) an isolated DNA fragment of SEQ ID NO:1 or SEQ ID NO:3;
 - (b) an isolated DNA fragment which, when hybridized for 60 minutes at 68°C in the presence of a hybridization buffer comprising sodium dodecyl sulfate, hybridizes to the complement of isolated DNA of (a) above and which encodes a TADG-12 protein; or
 - (c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein.
10. An isolated and purified TADG-12 protein of claim 9, wherein said TADG-12 protein consists of an amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4.
11. A method of detecting expression of TADG-12 mRNA, comprising the steps of:
 - (a) contacting mRNA obtained from a cell with a labelled hybridization probe, said probe having a nucleic acid sequence complementary to a TADG-12 mRNA encoding the TADG-12 protein of claim 9 or 10, said probe being configured to hybridize with said TADG-12 mRNA; and
 - (b) detecting hybridization of the probe with the mRNA.
12. An antibody that specifically binds to the TADG-12 protein of claim 9 or 10.
13. A method for diagnosing a cancer in an individual, comprising the step of:
detecting expression of the TADG-12 protein of claim 9 or 10 in a biological sample, wherein the presence of expression of said TADG-12 protein in said sample is indicative of the presence of a cancer in said individual, and wherein the absence of expression of said TADG-12 protein in said sample is indicative of the absence of a cancer in said individual.

14. The method of claim 13, wherein said biological sample is blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy, or circulating tumor cells.

15. The method of claim 13 or 14, wherein said detection of expression of the TADG-12 protein is by means of Western blot, ELISA sandwich assay, radioimmunoassay, or flow cytometry.

16. The method of claim 13, 14, or 15, wherein said cancer is ovarian cancer, breast cancer, lung cancer, colon cancer, or prostate cancer.

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

(a) isolating mRNA from said sample; and

(b) detecting TADG-12 mRNA encoding TADG-12 protein of claim 9 or 10 in said sample, wherein the presence of said TADG-12 mRNA in said sample is indicative of the presence of malignant hyperplasia, and wherein the absence of said TADG-12 mRNA in said sample is indicative of the absence of malignant hyperplasia.

18. The method of claim 17, wherein said detection of TADG-12 mRNA is by PCR amplification.

19. The method of claim 18, wherein said PCR amplification uses a primer sequence as set forth in SEQ ID NOs:28, 29, 30, or 31.

20. The method of claim 17, 18, or 19, wherein said biological sample is blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy, or circulating tumor cells.

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

(a) isolating protein from said sample; and

(b) detecting the TADG-12 protein of claim 9 or 10 in said sample, wherein the presence of said TADG-12 protein in said sample is indicative of the presence of

malignant hyperplasia, and wherein the absence of said TADG-12 protein in said sample is indicative of the absence of malignant hyperplasia.

22. The method of claim 21, wherein said detection is by immunoaffinity to an antibody, and wherein said antibody specifically binds to said TADG-12 protein.

23. The method of claim 21 or 22, wherein said biological sample is blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy, or circulating tumor cells.

24. An immunogenic composition, comprising (i) a TADG-12 protein of SEQ ID NO:2 or SEQ ID NO:4 or an immunogenic fragment of a TADG-12 protein consisting of an amino acid sequence shown in SEQ ID NO:8, 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 or 136 and (ii) an appropriate adjuvant.

25. Use of the immunogenic composition of claim 24 in the preparation of a medicament to vaccinate an individual against TADG-12.

26. The method of claim 11, wherein said probe hybridizes with the TADG-12 mRNA at about 42°C in the presence of 50% formamide, followed by a first wash at about 65°C with about 2 x SSC containing 1% SDS, and followed by a second wash at about 65°C with about 0.1 x SSC.

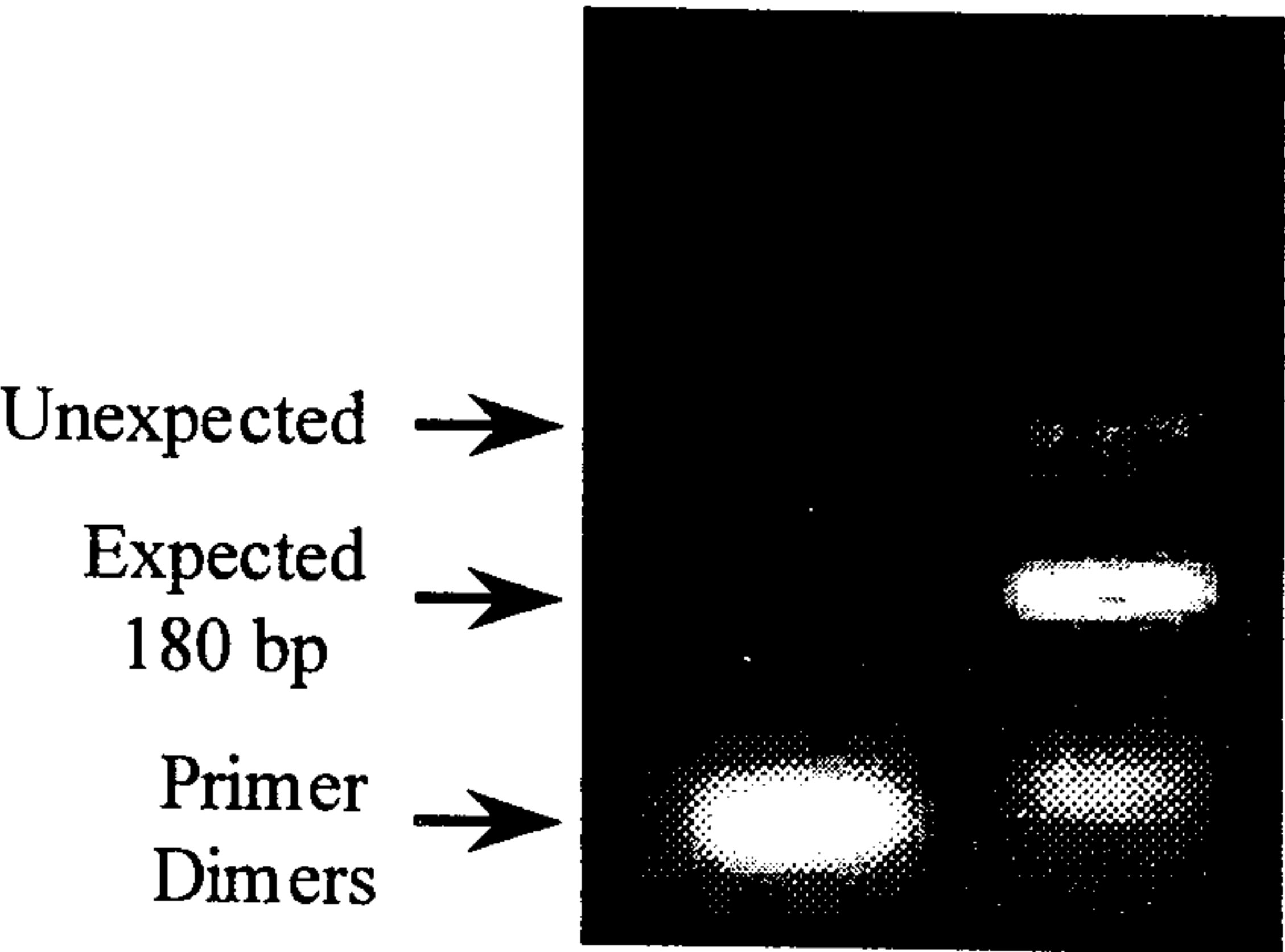


FIG. 1A

TADG12

↓

```

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61  CAGGTGGGTCTAGTTTCCCTGTTGGACAATCCAGCCCCATCCCCTTGGTGGAGAAGATT
   Q V G L V S L L D N P A P S H L V E K I

                                     (SEQ ID NO. 5)
121 GTCTACCACAGCAAGTACAAGCCAAAGAGGCTGGGCAACGACATCGCCCTCCTA
    V Y H S K Y K P K R L G N (D) I A L L
                                     (SEQ ID NO. 6)

```

TADG12-V

↓

```

1  GGGTGGTGACGGCGGCGCACTGTGTTTATGAGATTGTAGCTCCTAGAGAAAGGGCAGACA
   V V T A A H C V Y E I V A P R E R A D R

61  GAAGAGGAAGGAAGCTCCTGTGCTGGAGGAAACCCACAAAATGAAAGGACCTAGACCTT
   R G R K L L C W R K P T K M K G P R P S

121 CCCATAGCTAATTCCAGTGGACCATGTTATGGCAGATACAGGCTTGTACCTCCCCAAGTC
   H S * (SEQ ID NO. 8)

181 ATGGACCATCCAGGTGGGTCTAGTTTCCCTGTTGGACAATCCAGCCCCATCCCCTTGGT
241 GGAGAAGATTGTCTACCACAGCAAGTACAAGCCAAAGAGGCTGGGCAACGACATCGCCCT
301 CCTAATCACTAGTGCGGCCGCTGCAGG (SEQ ID NO. 7)

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FIG. 1B

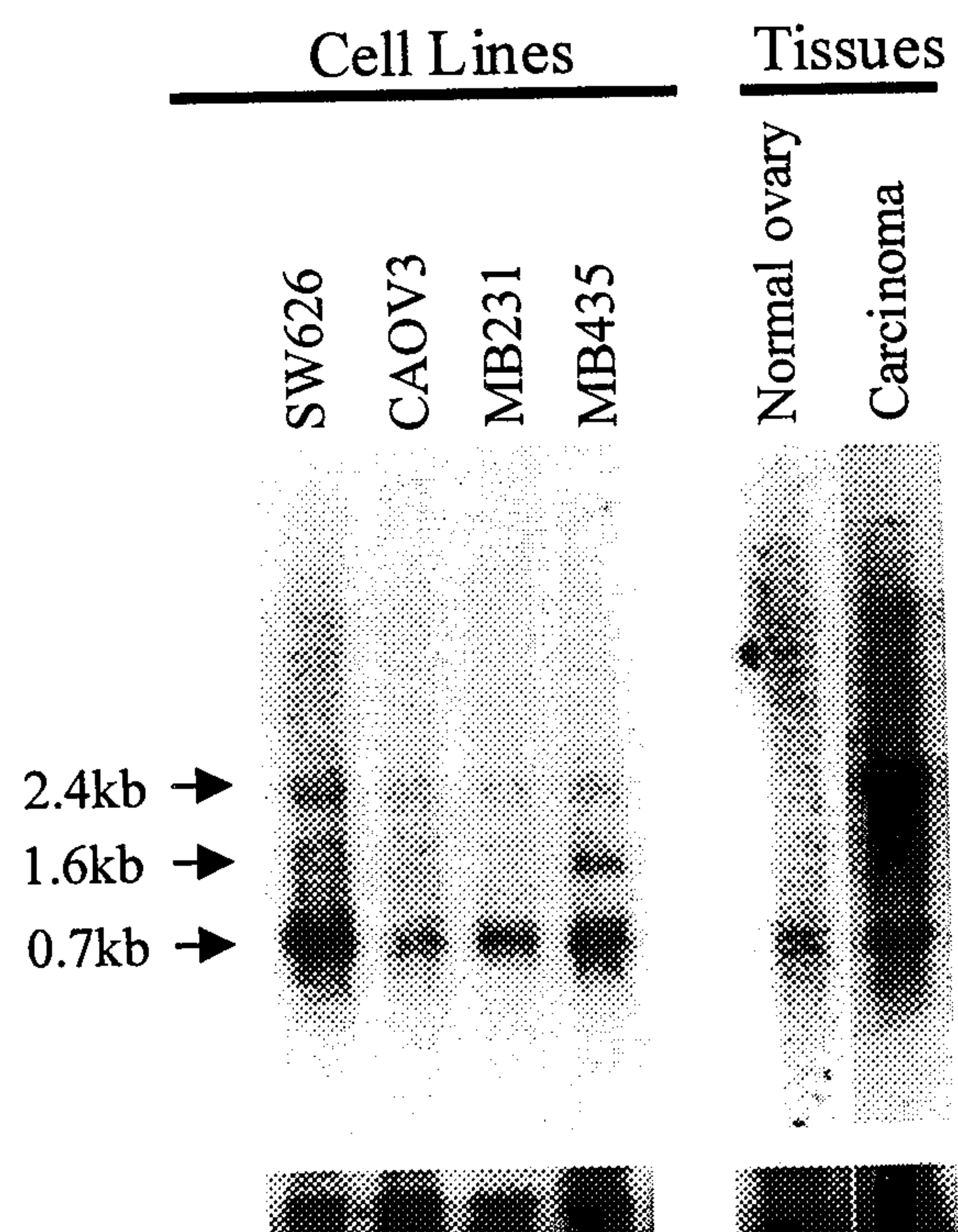


FIG. 2

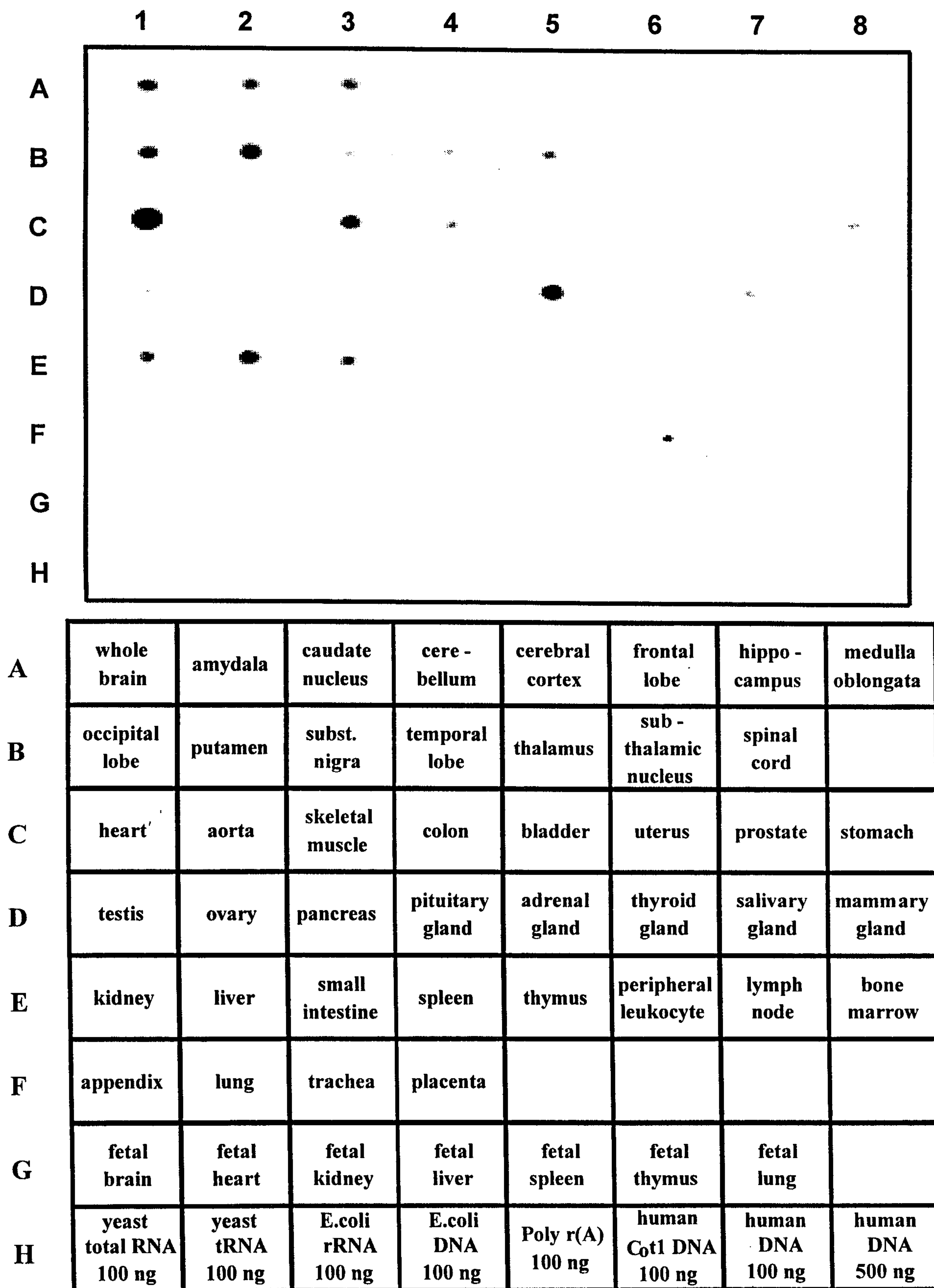


FIG. 3

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Compc8	CEG..FVC	AQTGRCVNRR	LLCNGDNDCG	DQSDEAN.C	(SEQ ID NO. 9)
Matr	CPG.QFTC	.RTGRCIRKE	LRCDGWADCT	DHSDELN.C	(SEQ ID NO. 10)
Gp300-1	CQQGYFKC	QSEGQCIPSS	WVCDQDQDCD	DGSDERQDC	(SEQ ID NO. 11)
Gp300-2	CSSHQITC	.SNGQCIPSE	YRCDHVRDCP	DGADE.NDC	(SEQ ID NO. 12)
TADG12	CSGK.YRC	RSSFKCIELI	TRCDGVSDCK	DGEDEYR.C	(SEQ ID NO. 13)
Tmprss2	CSNSGIEC	DSSGTCINPS	NWCDGVSHCP	GGEDENR.C	(SEQ ID NO. 14)
Cons	C	C	C	C	DE C

FIG. 5A

BovEntk	VRLVGGSGPH	EGRVEI.FHE	GQWGTVCDDR	WELRGGLVVC	RSLGYKGVQS
MacSR	VRLVGGSGPH	EGRVEI.LHS	GQWGTICDDR	WEVRVGQVVC	RSLGYPGVQA
TADG12	VRVGG...QN	AVLQVFTA..	ASWKTMCSD	WKGHYANVAC	AQLGFP.SYV
Tmprss2	VRLYG...PN	FILQMYSSQR	KSWHPVCQDD	WNENYGRAAC	RDMGYKNNFY
HumEntk	VRFFNGTTNN	NGLVRFRIQ.	SIWHTACAEN	WTTQISNDVC	QLLGLGSG..
Cons	VR		W	C	W C

BovEntk	VHKRAYFGKG	TGPIWLNEVF	CFGK..ESSI	EECRIRQWGV	R.ACSHDEDA
MacSR	VHKAHFGQG	TGPIWLNEVF	CFGR..ESSI	EECKIRQWGT	R.ACSHSEDA
TADG12	SSDNLRVSSL	EGQFREEFVS	I.DHLLPDDK	VTALHHSVYV	REGCASGHVV
Tmprss2	SSQGIVDDSG	STSEFMKLNTS	A.GNV...DI	YKKLYHS...	.DACSSKAVV
HumEntk	NSSKPIFSTD	GGPFVKLNTA	PDGHLILTPS	QQ.....	...CLQDSL
Cons					C

BovEntk	GVTCT	(SEQ ID NO. 15)
MacSR	GVTCT	(SEQ ID NO. 16)
TADG12	TLQCT	(SEQ ID NO. 17)
Tmprss2	SLRCL	(SEQ ID NO. 18)
HumEntk	RLQC.	(SEQ ID NO. 19)
Cons	C	

FIG. 5B

ProM	LWVLTAAHCK	KPNL	QVFLGKHNL	QRESSQEQSS	VVRAVIHPDY
Try1	QWVVSAGHCY	KSRI	QVRLGEHNIE	VLEGNEQFIN	AAKIIRHPQY
Kal	QWVLTAAHCF	D.GLPLQDVW	RIYSGILNLS	DITKDTFFSQ	IKEIIHQNY
TADG12	LWIITAACHV	.YDLYLPKSW	TIQVGLV..S	LLDNPAPSHL	VEKIVYHSKY
Tmprss2	EWIVTAAHCV	EKPLNNPWHW	TAFAGILRQS	FMFYGA.GYQ	VQKVISHPNY
Heps	DWVLTAAHCF	PERNRVLSRW	RVFAGAVAQA	SPHGLQLG..	VQAVVYHGGY
Cons	W	A HC	G		H Y
ProM	DAAS	HDQDIMLLRL	ARPAKLSELI	QPLPLERDCS	ANT..TSCHI
Try1	DRKT	LNNDIMLIKL	SSRAVINARV	STISLPTAPP	ATG..TKCLI
Kal	KVSE	GNHDIALIKL	QAPLNYTEFQ	KPICLPSKGD	TSTIYTNCWV
TADG12	KPKR	LGNDIALMKL	AGPLTFNEMI	QPVCLPNSEE	NFPDGKVCWT
Tmprss2	DSKT	KNNDIALMKL	QKPLTFNDLV	KPVCLPNPGM	MLQPEQLCWI
Heps	LPFRDPNSEE	NSNDIALVHL	SSPLPLTEYI	QPVCLPAAGQ	ALVDGKICTV
Cons		DI L L		L	C
ProM	LGWGKTAD..	GDFPDTIQCA	YIHLVSREEC	EHA..YPGQI	TQNMLCAGDE
Try1	SGWGNTASSG	ADYPDELQCL	DAPVLSQAKC	EAS..YPGKI	TSNMFCVGFL
Kal	TGWGFSKEK.	GEIQNILQKV	NIPLVTNEEC	QKR.YQDYKI	TQRMVCAGYK
TADG12	SGWGAT.EDG	GDASPVLNHA	AVPLISNKIC	NHRDVYGGII	SPSMLCAGYL
Tmprss2	SGWGAT.EEK	GKTSEVLNAA	KVLLIETQRC	NSRYVYDNLI	TPAMICAGFL
Heps	TGWGNT.QYY	GQQAGVLQEA	RVPIISNDVC	NGADFYGNQI	KPKMFCAGYP
Cons	GWG		C	I	M C G
ProM	KYGKDSCQGD	SGGPLVC	(SEQ ID NO. 20)		
Try1	EGGKDSCQGD	SGGPVVC	(SEQ ID NO. 21)		
Kal	EGGKDACKGD	SGGPLVC	(SEQ ID NO. 22)		
TADG12	TGGVDSCQGD	SGGPLVC	(SEQ ID NO. 23)		
Tmprss2	QGNVDSCQGD	SGGPLVT	(SEQ ID NO. 24)		
Heps	EGGIDACQGD	SGGPFVC	(SEQ ID NO. 25)		
Cons	D C GD	SGGP V			

FIG. 5C

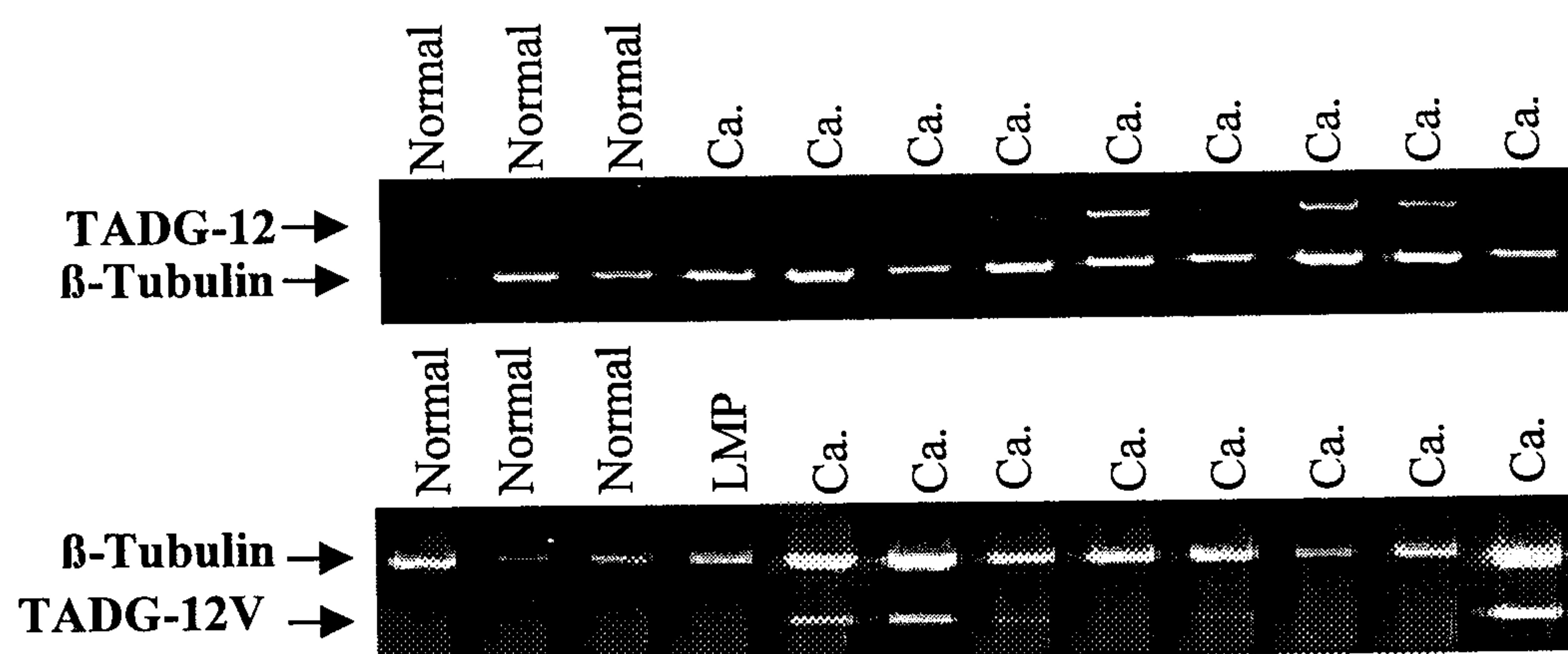


FIG. 6



FIG. 7A

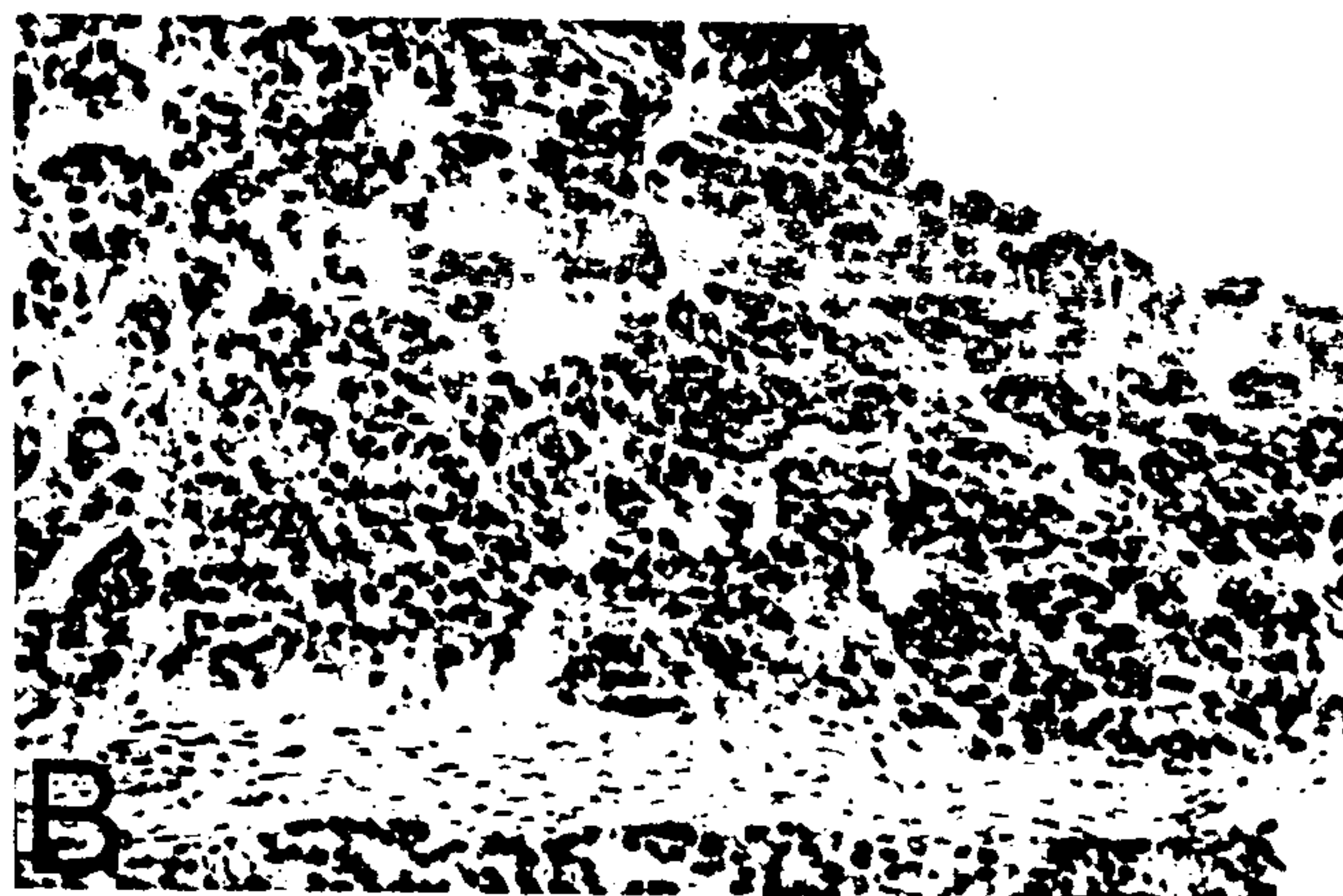


FIG. 7B



FIG. 7C

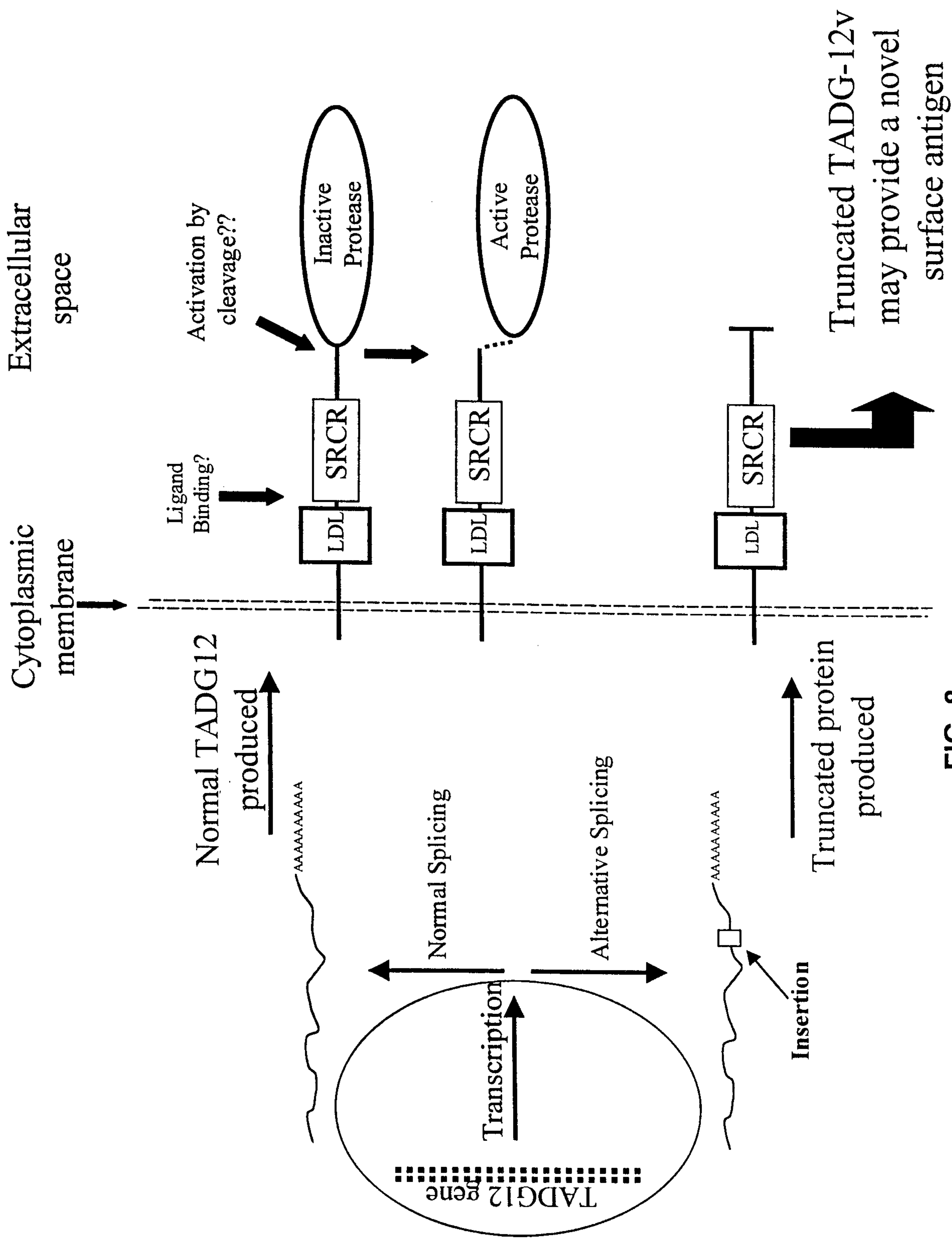


FIG. 8