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(19) **United States**(12) **Patent Application Publication**
O'Brien et al.(10) **Pub. No.: US 2010/0291124 A1**(43) **Pub. Date: Nov. 18, 2010**(54) **EXTRACELLULAR SERINE PROTEASE**

is a continuation-in-part of application No. 08/915,659, filed on Aug. 21, 1997, now Pat. No. 7,014,993.

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Houston, TX 77071 (US)(21) Appl. No.: **12/802,335**(22) Filed: **Jun. 4, 2010****Related U.S. Application Data**

(60) Division of application No. 10/652,846, filed on Aug. 29, 2003, now Pat. No. 7,732,163, which is a continuation-in-part of application No. 09/796,294, filed on Feb. 28, 2001, now Pat. No. 7,157,084, which is a continuation-in-part of application No. 09/618,259, filed on Jul. 18, 2000, now Pat. No. 6,642,013, which is a continuation-in-part of application No. 09/137,944, filed on Aug. 21, 1998, now Pat. No. 7,067,250, which

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(52) **U.S. Cl. 424/185.1; 435/7.1; 530/350; 506/9; 435/7.92; 435/6; 435/375; 536/23.1**(57) **ABSTRACT**

The present invention provides a DNA encoding a novel extracellular serine protease termed Tumor Antigen Derived Gene-14 (TADG-14) which is overexpressed in ovarian, breast and colon carcinoma samples. Also provided are vector and host cells capable of expressing the DNA of the present invention, as well as the uses of the DNA and protein of the present invention. Also provided is a TADG-14 protein variant that has a potential role for detecting and targeting of ovarian carcinomas.

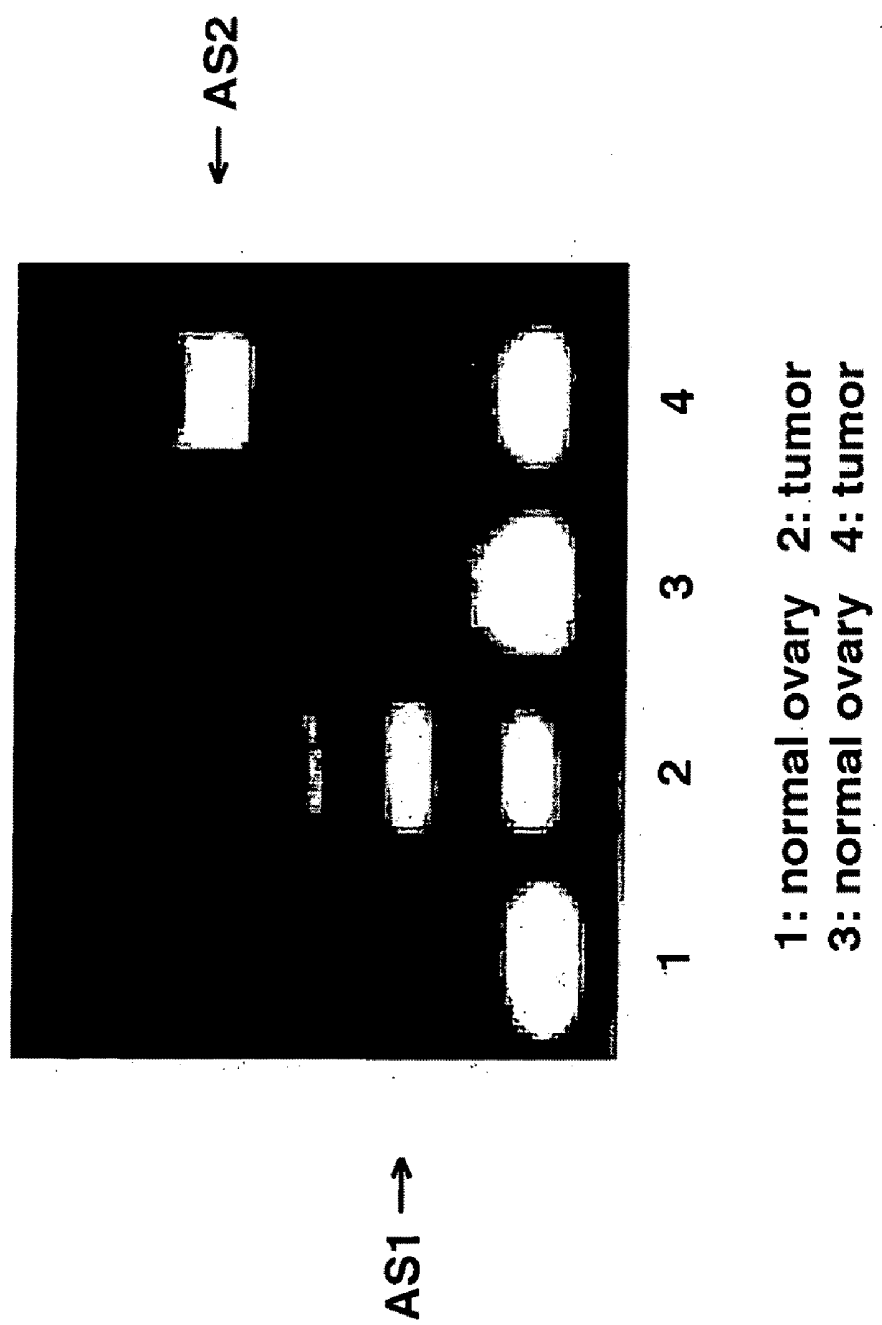


FIG. 1

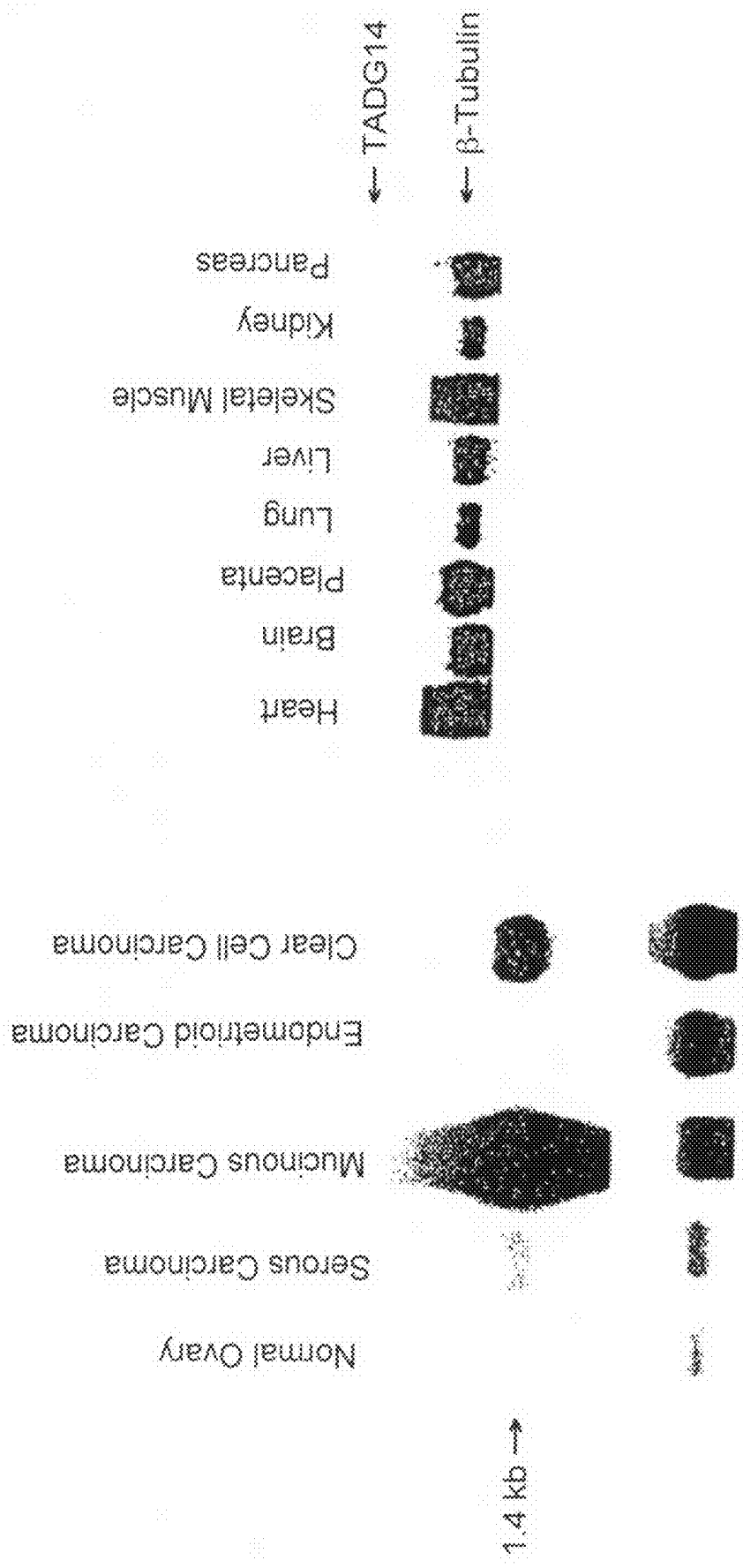
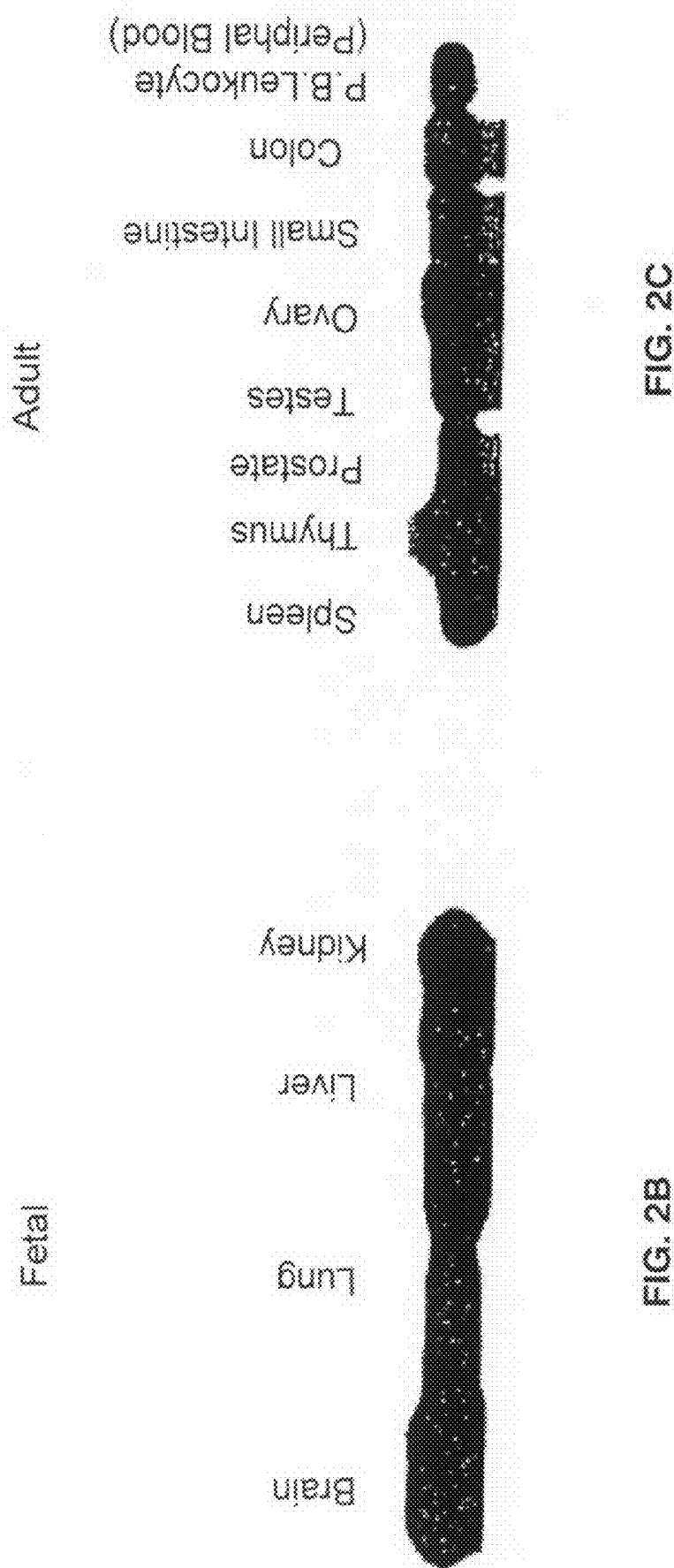


FIG. 2D

FIG. 2A



1 CTGTAGCAGGAGCTTACCAAGTCTCTCCGAACCTCAAAATGGAAGAAATACCTTATGAATGTAAAGATGTAGGGGGTCA 80
81 TGGCTTGTAATTTACACAGTGTAAATGAAACCATCTAGAGGATTATGAGGAATCCTTTCTATGTGATTTTCAATCATAG 160
161 CAAGCAAGAAAGGCTCCAGTGTCAAGGATGTTTCAAGGATATAAACAAGTCCATATTTGACATATAAACAAGTCCATATTTCAATCATAG 240
241 GATCTGAGTGAATGTGAAGCAATGTTTCAAGGATATAAACAAGTCCATATTTGACATATAAACAAGTCCATATTTCAATCATAG 320
321 CTTCAAAATGAAAGTTACGTAATCAACCATCAAGAAAGTTCATGTTTGGTAAATTTCTGTTTACTAGAAATGTAGGAAAGG 400
401 GTATAGCTTTGAATCCCAATACACATTTGTCAGTGGGAAACATAAGGCTCCCAACAGGCAAAATTCAGGAGGATAGGT 480
481 TTCAGGGAATGCCCTGGATTCTGGAAGACCTCACCATGGGACGCCCGACCTCGTGGCGGCAAGACGTGGATGTTCTCTG 560
561 CTCTTGCTGGGGAGCCTGGCAGGACACTCCAGGGCACAGGAGGACAAAGTGTCTGGGGGTTCATGATGCCAACCCCA 640
641 L L L G G A W A G H S R A Q E D K V L G G H E C Q P H 720
721 S Q P W Q A A L F Q G Q Q L L C G G V L V G G N W V L 800
801 T A A H C K K P K Y T V R L G D H S L Q N K D G P E 880
881 Q E I P V V Q S I P H P C Y N S S D V E D H N H D L M 960
961 L L Q L R D Q A S L G S K V K P I S L A D H C T Q P G 1040
1041 Q K C T V S G W G T V T S P R E N F P D T L N C A E 1120
1121 V K I F P Q K K C E D A Y P G Q I T D G M V C A G S S 1200
1201 K G A D T C Q G D S G G P L V C D G A L Q G I T S W G 1280
1281 S D P C G R S D K P G V Y T N I C R Y L D W I K K I 7
I G S K G * SEQ ID NO. 6

— = Kozak's Consensus sequence

+ = Conserved amino acids of catalytic triad H, D, S

NSS = Possible N - linked glycosylation site

— = Poly - adenylation signal

— = Conserved nt of catalytic triad

— = aa required for formation of an oxyanion hole for catalytic activity

FLL = Secretion signal sequence

Fig. 3A

hbk2	-----MW	FLVLCIAISL	GGTGAAPPIQ	SRIVGGWECE	OHSQPNQAAAL	42
hPSA	-----MW	VPMVFETLSV	TWIGAAPLIL	SRIVGGWECE	KHSQPNQVLV	42
mNeur	MGRPPPCAIO	PWILILIFMG	AWAGLTRAQG	SKILEGREGI	PHSQPNQAAAL	50
htADG14	MGRPPRRAAK	TWMFILLLEG	AWAGHSRAQE	DKVLGGHECQ	PHSQPNQAAAL	50
hProm	-----MKK	LMVVSIIAA	AWA...EEQ	NKLHGCGPCD	KTSHPYQAAL	39
hbk2	YHSTFQCGG	IELVHROWVLT	AAACISDNQO	LWIGRHNLFD	DENTAQFVHV	92
hPSA	ASRCRAVCCG	VELVHPQWVLT	AAHCIRNKSV	ILIGRHSLFPH	PEDTGOVFQV	92
mNeur	FOGERNLCGG	VLVGGDRWVLT	AAHCKKQKYS	VRIGDHSIQS	RDQPEGEIQV	100
htADG14	FOGQQLCCG	VLVGGGNVLT	AAHCKKPKVT	VRIGDHSIQN	KDGPPEQEIPV	100
hProm	YTSCHLLCCG	VLIIHPLNVLT	AAHCKKPNLQ	VFLGKHNLRO	RESSQEQSSV	89
hbk2	SESPHHPGPN	MSLLENHTRO	ADEDYSHDLM	LLRITTEPADT	ITDAVKVVEL	142
hPSA	SHSEPHHLID	MSLLKNRFLR	PCDDSSHDLM	LLRSEPAE.	ETDAVKVMDE	141
mNeur	AQSIQHBCCN	NS.....	NPEDHSHDLM	LIRLQNSAN.	LGDKKWPVQL	141
htADG14	VOSIHPHCYN	SS.....	DVEDHSHDLM	LIQERDOAS.	LGSKKWPISL	141
hProm	VRAVIHPD.		DAASHDQSIIM	LIQLAREPAK.	LSELIQELPE	127
hbk2	PTQEPFVGSST	CLASGNGSIE	PENFSEPDDE	QCVDLKLILPN	DECEKAHVQK	192
hPSA	PTQEPALGTT	CYASGNGSIE	PEEFLTPKKI	QCVDLHVISN	DVCAQVHROK	191
mNeur	ANLCFKVQOK	CIISGNGIVT	SPOENFNTLE	NCAEVKIYSQ	NKGERAYPGK	191
htADG14	ADHCTOPGOK	CIIVSGNGIVT	SPRENFPSTL	NCAEVKIIPPQ	KKCEDAIPGQ	191
hProm	ERDCSANTTS	CHILGNGKTA	D..GDPPDHI	OCAYIHLVSR	ECEHAYPGQ	175
hbk2	VTDFMICVGH	LEEGKDTICVG	DSGGPLMCBG	VLOGVTSWGY	VPCGTPNKPS	242
hPSA	VTKFMICAGR	WTGKSTICSG	DSGGPLVNCG	VLOGITSWGS	EPCALPERES	241
mNeur	ITEGVVCAGS	SN.GADTCQG	DSGGPLVNCBG	MLOGITSWGS	DPCGKPEKPG	240
htADG14	ITDGNVVCAGS	SK.GADTCQG	DSGGPLVNCBG	ALOGITSWGS	DPCGERSDNG	240
hProm	ITQNNMLCAGD	EKYGKIDSCQG	DSGGPLVCGD	HIRGLVSWGN	IFCGSKKPKG	225
hbk2	WAVRULSVVK	WIEDTIAENS	SEQ ID NO: 9			262
hPSA	LATKVVHVRK	WINDIIVANP	SEQ ID NO: 10			261
mNeur	WYKICRVTIT	WIKKMDNRD	SEQ ID NO: 8			260
htADG14	WYINICRYLD	WERRIICSKG	SEQ ID NO: 7			260
hProm	WYINVCRTYN	WOKIIOAK-	SEQ ID NO: 11			244

Fig. 3B

201 Prom WVLTA~~AHC~~ KK PNLQV...F LGKHNLRQRE SSQEQSSVVR AVIHPDY... 250
 Tag14 WVWTA~~AHC~~ KK PKYTV...R LGDHSLO~~ND~~ GPEQEI~~PVVQ~~ SIPHPCY...
 Try1 WVVSAGHC YK SRIQV...R LGEHNIEVLE GNEQFINAAK IIRHPQY...
 Scce WVLTA~~AHC~~ KM NEYTV...H LGSDTLGDRR A..QRIKASK SFRHPGY...
 Heps WVLTA~~AHC~~ FP ERNRVLSRWR VFAGAVAQAS PHGLGLGVQA VVYHGGYLF 250

251 Prom ...DAASHDQ DIMLL RLARP AKLSELIQPL PLERDCSA.. NTTSCHILGW
 Tag14 NSSDVEDHNH DLMLL QLRDQ ASLGSVKVPI SLADHCTQ.. PGQNC~~TVSGW~~
 Try1 ...DRKTLNN DIMLL KLSSR AVINARVSTI SLPTAPPA.. TGTKCLISGW
 Scce ST...QTHVN DLM~~LV~~ K~~LN~~SQ ARLSSMVKKV RLPSRCEP.. PGTTC~~TVSGW~~
 Heps RDPNSEENSN DIALV HLSSP LPLTEYIQPV CLPAAAGQALV DGKIC~~TVTGW~~ 300

301 Prom GKTAD..GDF PDTIQCAYIH LVSREECEHA ..TPGQITQN MLCAGDEKYG 350
 Tag14 GTVTSPRENF PDTLNC~~AEVK~~ IFPQKKCEDA ..YPGQITDG MVCAGSSK.G
 Try1 GNTASSGADY PDELQCLDAP VLSQAKCEAS ..YPGKITSN MFCVGFLEGG
 Scce GTTTS~~PDVTF~~ PSDLMCVDVK LISPQDCTKV ..YKDLLENS MLCAGIPDSK
 Heps GNTQYYGQQ. AGVLQEARVP IISNDVCNGA DFGNQIKPK MFCAGYPEGG 350

351 Prom KDSCQ GDSGG SEQ ID No. 1
 Tag14 ADTCQ GDSGG SEQ ID No. 2
 Try1 KDSCQ GDSGG SEQ ID No. 3
 Scce KNACN GDSGG SEQ ID No. 4
 Heps IDACQ GDSGG SEQ ID No. 5
 PRIMER

Fig. 4

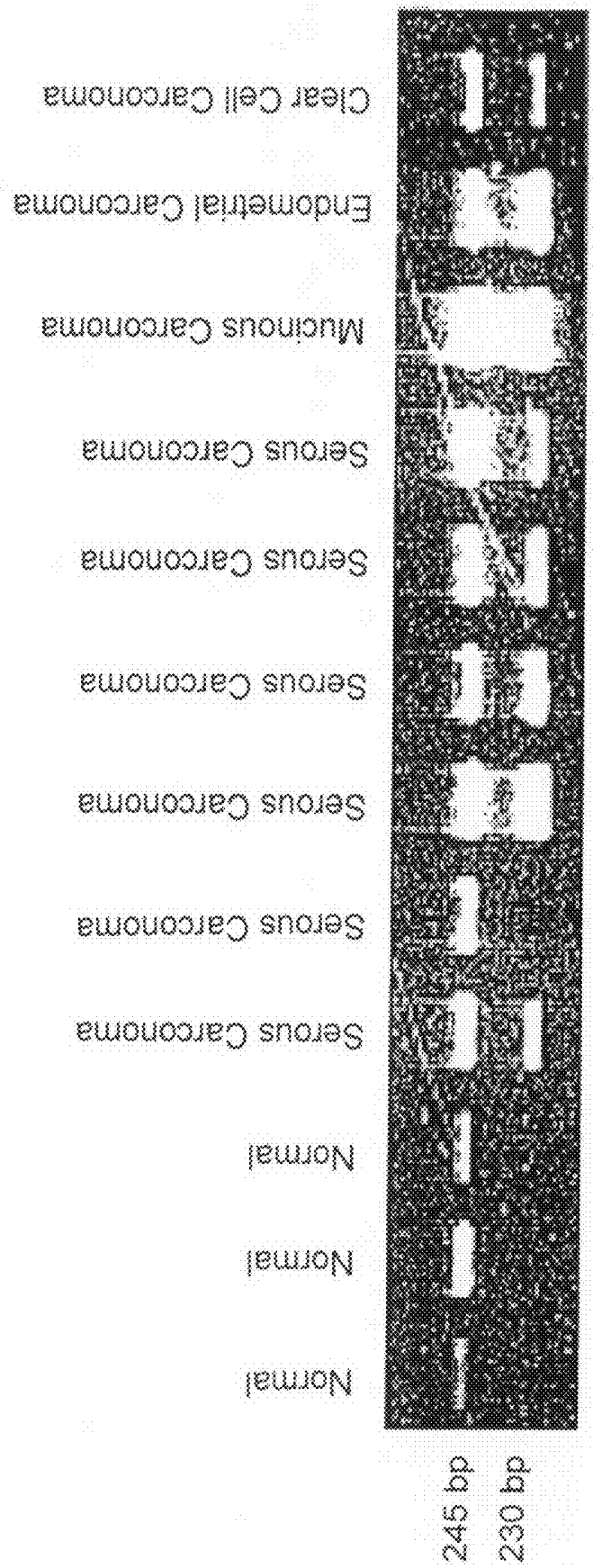


FIG. 5A

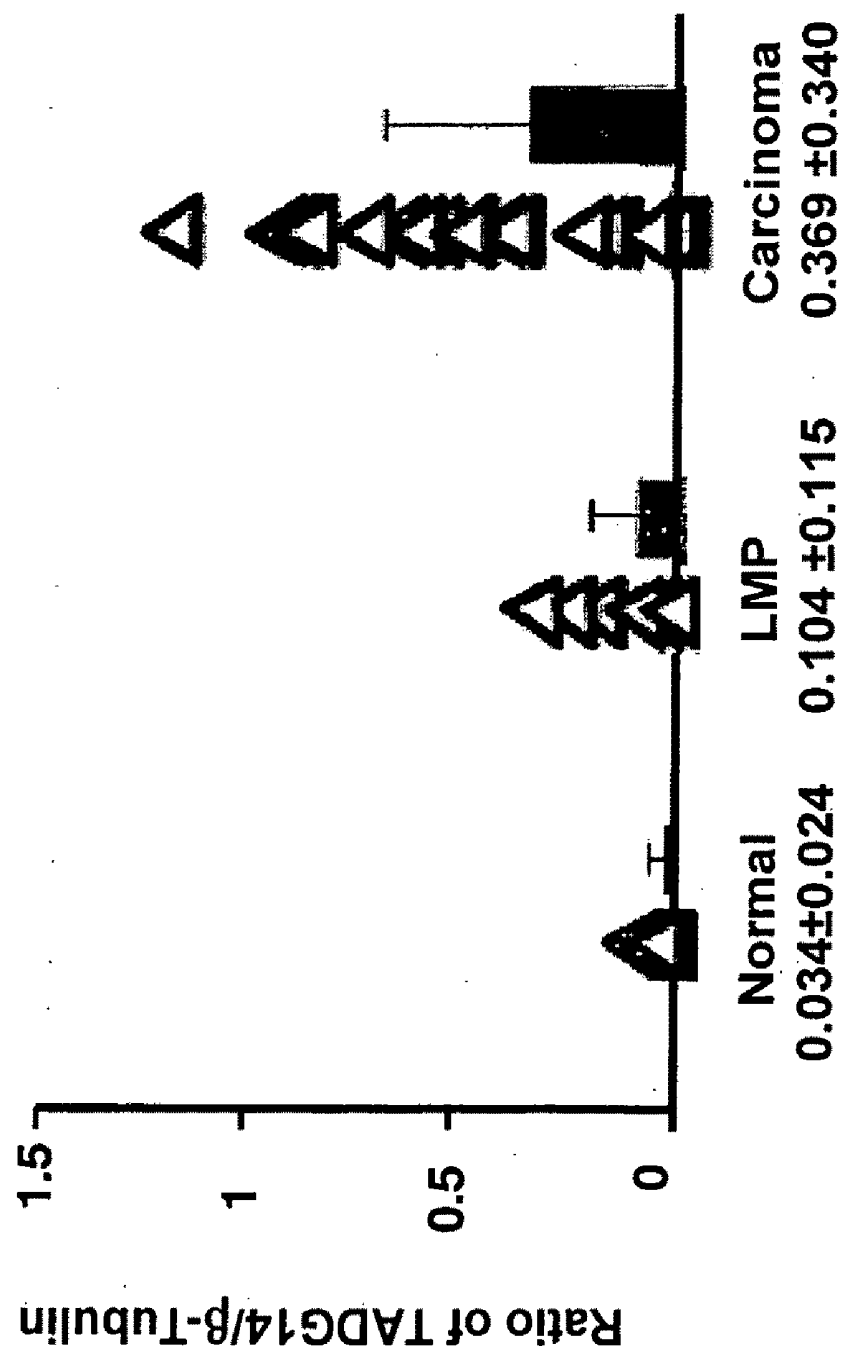


FIG. 5B

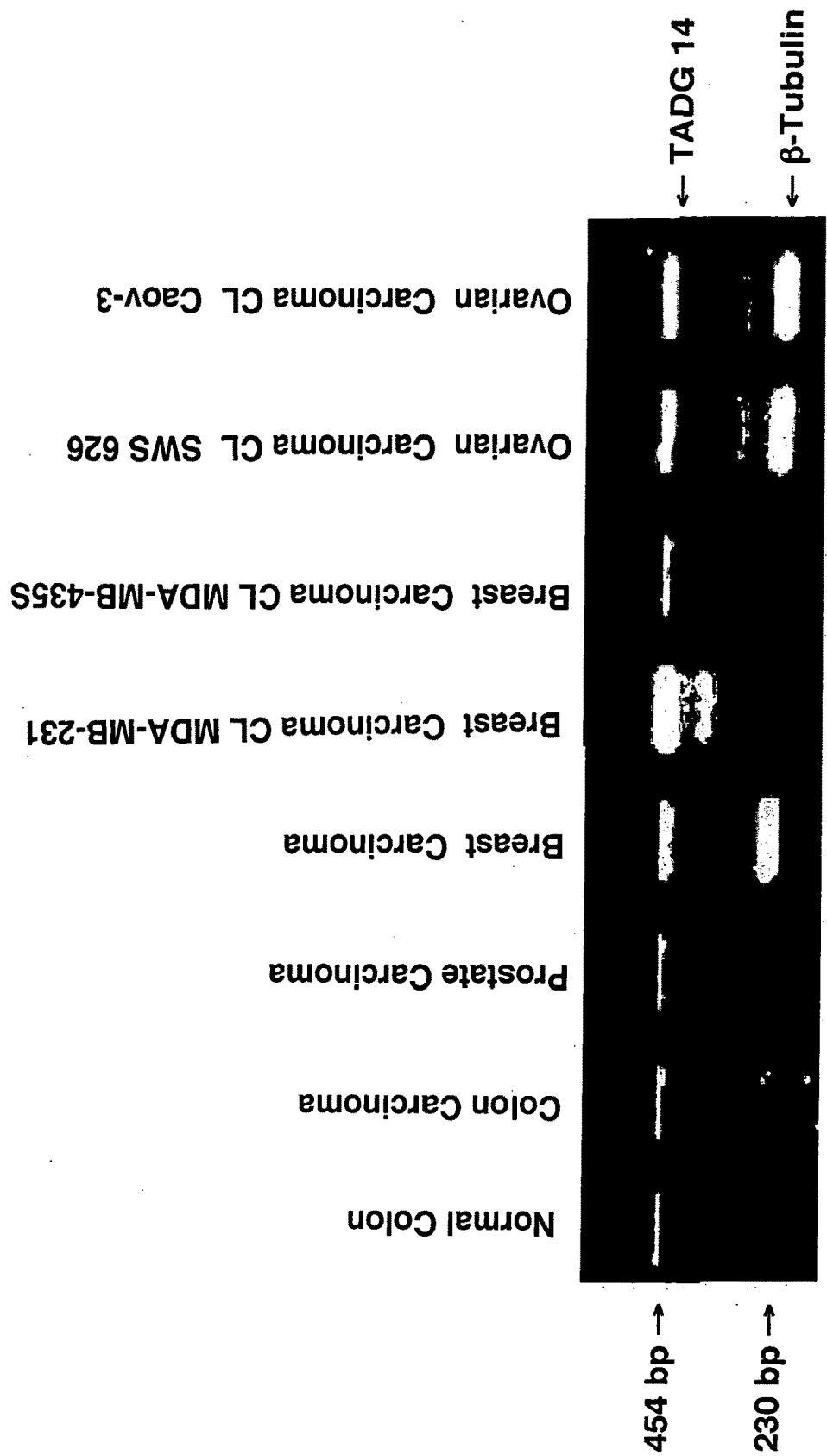


FIG. 6

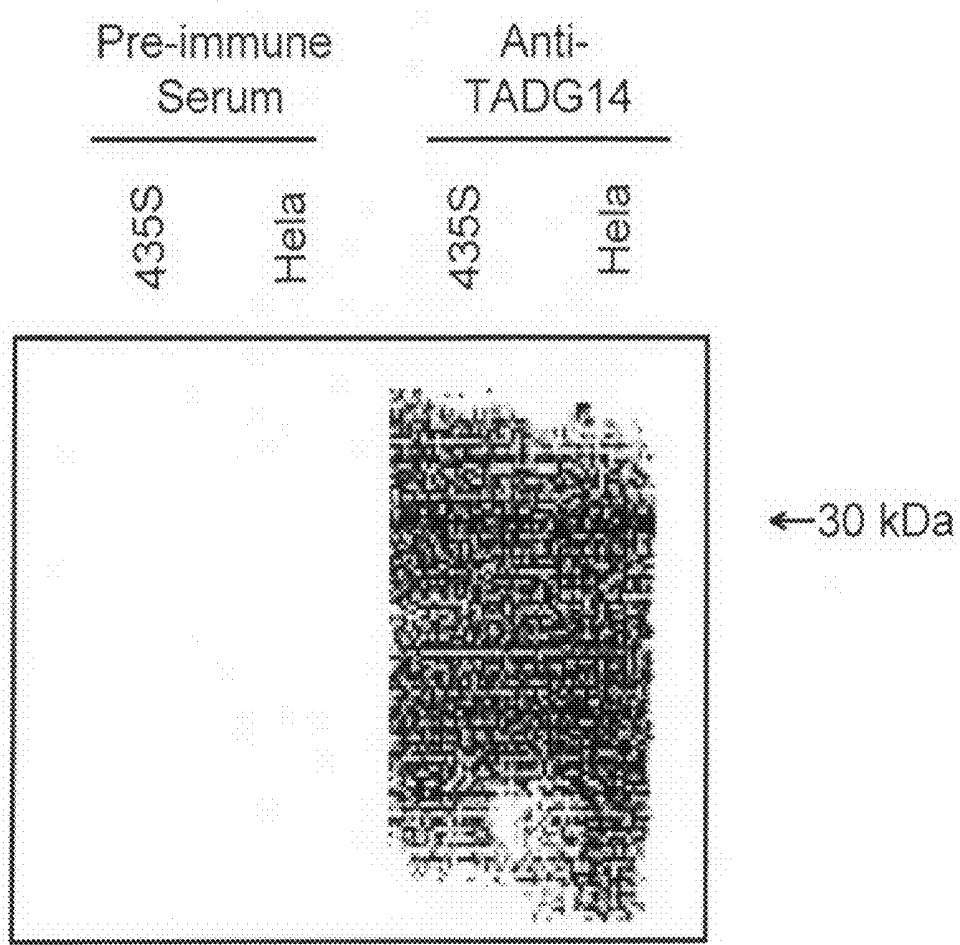


FIG. 7

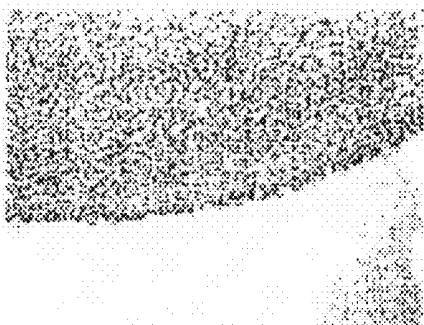


FIG. 8A



FIG. 8D

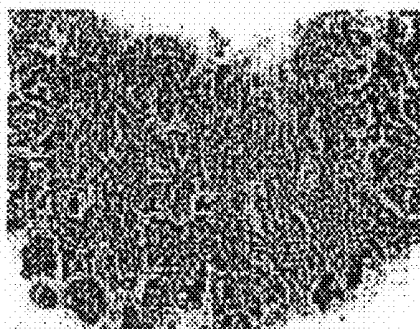


FIG. 8B

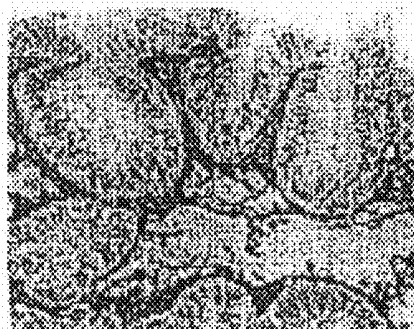


FIG. 8E

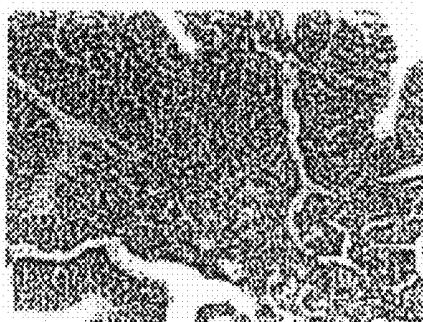


FIG. 8C



FIG. 8F

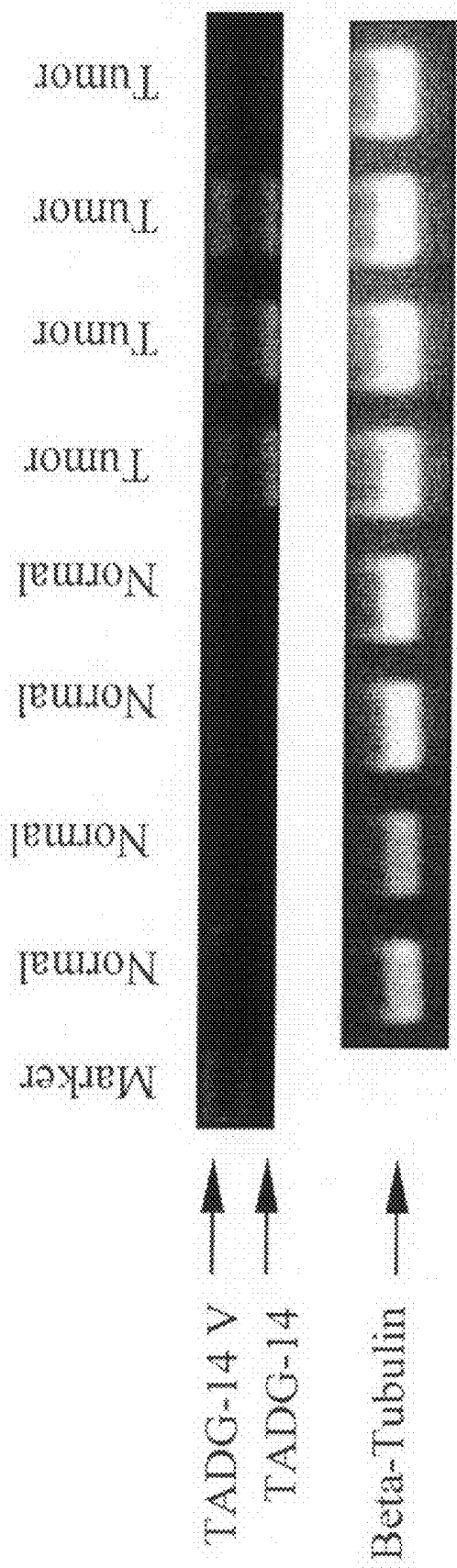


FIG. 9

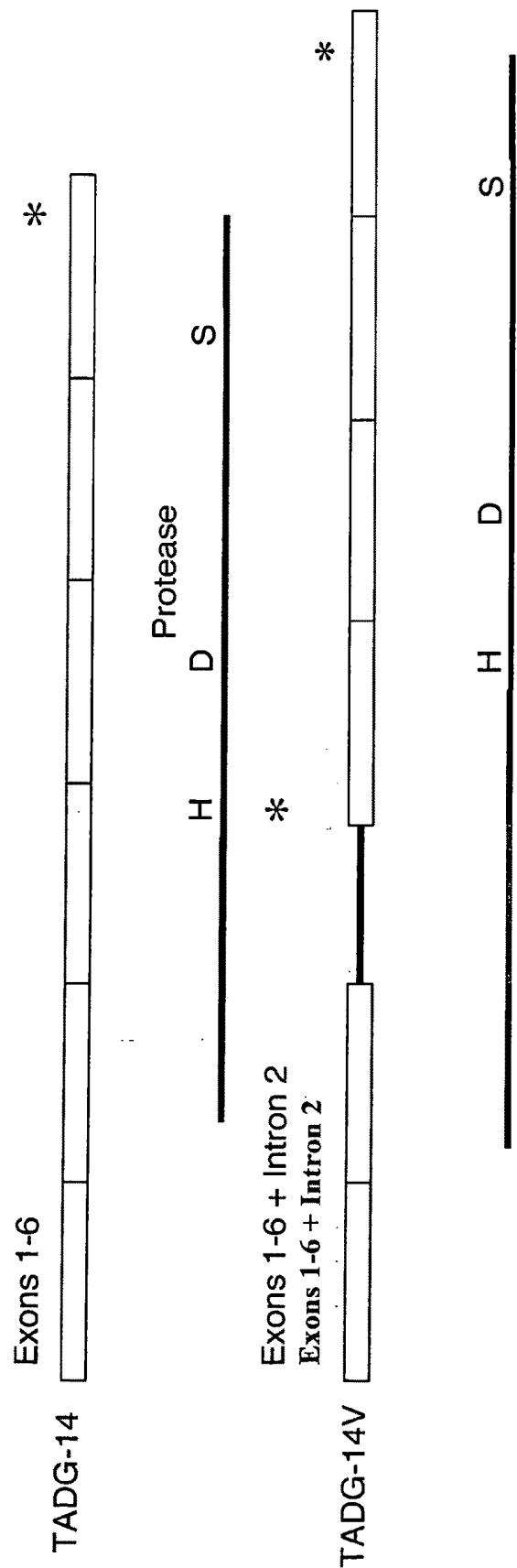


FIG. 10

Tadg 14	MGRPRPRAAK	TWMFLLLLGG	AWA.....			CVHLNPQWPS
Tadg 14 V	MGRPRPRAAK	TWMFLLLLGG	AWAACGSDDL	LTkLYAENLP		
Tadg 14		GH	SRAQEDKVLG	GHECQPHSQP	WQAALFQGQQ	
Tadg 14 V	QPSHCPRGWR	SNPLPPAAGH	SRAQEDKVLG	GHECQPHSQP	WQAALFQGQQ	
Tadg 14	LLCGGVLVGG	NWVLTAAHCK	KPKYTVRLGD	HSLQNKDGPE	QEIPVVQSIP	
Tadg 14 V	LLCGGVLVGG	NWVLTAAHCK	KPKYTVRLGD	HSLQNKDGPE	QEIPVVQSIP	
Tadg 14	HPCYNSSDVE	DHNHDLMLLQ	LRDQASLGSK	VKPISLADHC	TQPGQKCTVS	
Tadg 14 V	HPCYNSSDVE	DHNHDLMLLQ	LRDQASLGSK	VKPISLADHC	TQPGQKCTVS	
Tadg 14	GWGTVTSPRE	NFPDTLNCAE	VKIFPQKKCE	DAYPGQITDG	MVCAGSSKGA	
Tadg 14 V	GWGTVTSPRE	NFPDTLNCAE	VKIFPQKKCE	DAYPGQITDG	MVCAGSSKGA	
Tadg 14	DTCQGD\$GGP	LVCdGALQGI	TSWGSDPCGR	SDKPGVYTNI	CRYLDWIKKI	
Tadg 14 V	DTCQGD\$GGP	LVCdGALQGI	TSWGSDPCGR	SDKPGVYTNI	CRYLDWIKKI	
Tadg 14	IGSKG (SEQ ID NO. 7)					
Tadg 14 V	IGSKG (SEQ ID NO. 75)					

FIG. 11

EXTRACELLULAR SERINE PROTEASE**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This patent application is a divisional of and claims benefit of priority under 35 U.S.C. 120 of pending U.S. Ser. No. 10/652,846, filed Aug. 29, 2003, which is a continuation-in-part under 35 U.S.C. 120 of U.S. Ser. No. 09/796,294, filed Feb. 28, 2001, now U.S. Pat. No. 7,157,084, which is a continuation-in-part under 35 U.S.C. 120 of U.S. application Ser. No. 09/618,259, filed Jul. 18, 2000, now U.S. Pat. No. 6,642,013, which is a continuation-in-part under 35 U.S.C. 120 of U.S. application Ser. No. 09/137,944, filed Aug. 21, 1998, now U.S. Pat. No. 7,067,250, which is a continuation-in-part under 35 U.S.C. 120 of U.S. application Ser. No. 08/915,659, filed Aug. 21, 1997, now U.S. Pat. No. 7,014,993, the entirety of all of which hereby are incorporated reference.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention relates generally, to the fields of cellular biology and the diagnosis of neoplastic disease. More specifically, the present invention relates to a novel extracellular serine protease termed Tumor Antigen Derived Gene-14 (TADG-14).

[0004] 2. Description of the Related Art

[0005] Serine proteases comprise a family of protein degrading enzymes that serve a host of biological functions including activation of blood coagulation cascades, activation of growth and angiogenic factors and degradation of extracellular matrix components (1-4). In recent years, aberrant expression of serine proteases, such as plasminogen activator have been shown to correlate positively with the invasiveness and metastatic potential of tumor cells (3, 5-6). Presumably, this occurs by increasing the ability of the tumors to degrade extracellular matrix components either directly or indirectly through the proteolytic activation of other zymogenic proteases. More significantly, the serine protease known as the prostate specific antigen (PSA) has been used successfully as a tumor marker for the early diagnosis of prostate cancer due to its abnormal prevalence in the peripheral blood of these patients (7). Serine proteases play important roles in the cascade of events involved in the malignant process, and at least for prostate cancer, they provide sufficient signal to allow detection of early disease.

[0006] The prior art is deficient in the lack of effective means of screening to identify proteases overexpressed in carcinoma. The present invention fulfills this longstanding need and desire in the art.

SUMMARY OF THE INVENTION

[0007] The present invention discloses a 1343 base pairs long TADG-14 cDNA (SEQ ID No: 6) which encodes a 260 amino acid protein (SEQ ID No: 7) overexpressed in carcinoma. The availability of the TADG-14 gene opens the way for a number of studies that can lead to various applications.

[0008] In one embodiment of the present invention, there is provided a DNA encoding a TADG-14 protein having the amino acid sequence of SEQ ID NO. 7, a vector capable of expressing the DNA of the present invention, as well as host cell transfected with the vector that express the TADG-14 protein. Additionally embodied is a vector in which the

TADG-14 DNA is positioned in reverse orientation relative to the regulatory elements such that a TADG-14 antisense DNA is produced.

[0009] In another embodiment of the present invention, there is provided a DNA encoding a TADG-14 variant protein having the amino acid sequence of SEQ ID NO. 75 or fragments thereof, a vector capable of expressing said DNA, as well as host cell transfected with the vector that express the TADG-14 variant protein. The TADG-14 protein variant has a potential role for detecting and targeting of ovarian carcinomas.

[0010] The present invention also provides an isolated and purified TADG-14 protein (SEQ ID No: 7) and an isolated and purified TADG-14 variant protein (SEQ ID No: 75) or fragments of either protein. The present invention also provides antibodies or antibody fragments specific for the TADG-14 protein or the TADG-14 variant protein.

[0011] In another embodiment of the present invention, there are provided methods of using oligonucleotide probe, antibody or antibody fragments to detect TADG-14 mRNA, TADG-14 variant mRNA, TADG-14 protein, or TADG-14 variant protein in a biological sample. Generally, the sample is a biological sample from blood, interstitial fluid, ascites fluid, tumor tissue biopsy or circulating tumor cells. Preferably, the biological sample is from an individual; and typically, the individual is suspected of having cancer.

[0012] The present invention also provides kits for detecting TADG-14 mRNA, TADG-14 variant mRNA, TADG-14 protein, or TADG-14 variant protein. The kits comprises oligonucleotide probe, antibody or antibody fragments specific for TADG-14 or TADG-14 variant. The kits can further comprise a label for detecting the probe or antibody.

[0013] In yet another embodiment of the present invention, there is provided methods of inhibiting expression of TADG-14 in a cell with TADG-14 antisense DNA or TADG-14-specific antibody. Generally, the inhibition of TADG-14 expression is for treating cancer.

[0014] In another embodiment of the present invention, there is provided a method of targeted therapy to an individual, comprising the step of: (a) administering a compound containing a targeting moiety and a therapeutic moiety to an individual, wherein the targeting moiety is specific for TADG-14.

[0015] In another embodiment of the present invention, there are provided methods of diagnosing cancer in an individual through the detection of TADG-14 or TADG-14 variant at the protein or DNA level.

[0016] In yet another embodiment of the present invention, there is provided a method of vaccinating an individual against TADG-14 protein, comprising the step of (a) inoculating an individual with a TADG-14 protein or fragment thereof which lacks TADG-14 protease activity. Typically, inoculation with the TADG-14 protein or fragment thereof elicits an immune response in the individual, thereby vaccinating the individual against TADG-14. Generally, the individual has cancer, is suspected of having cancer or is at risk of getting cancer. Preferably, the TADG-14 fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 17, 18, 41, 42, 47, 48, 53, 56, or 64.

[0017] In another embodiment of the present invention, there is provided a method of producing activated immune cells directed toward TADG-14, comprising the steps of exposing immune cells to a TADG-14 protein or fragment

thereof which lacks TADG-14 protease activity. Usually, exposure to the TADG-14 protein or fragment thereof activates the immune cells, thereby producing activated immune cells directed toward TADG-14. Generally, the immune cells are B cells, T cells or dendritic cells. Preferably, the dendritic cells are isolated from an individual prior to exposure to a TADG-14 protein or fragment thereof, and then reintroduced into the individual subsequent to the exposure. Typically, the individual has cancer, is suspected of having cancer or is at risk of getting cancer. Preferably, the TADG-14 fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 17, 18, 41, 42, 47, 48, 53, 56, or 64.

[0018] In another embodiment of the present invention, there is provided an immunogenic composition, comprising an immunogenic fragment of a TADG-14 protein and an appropriate adjuvant. Preferably, the TADG-14 fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 17, 18, 41, 42, 47, 48, 53, 56, or 64.

[0019] In another embodiment of the present invention, there is provided an oligonucleotide having a sequence complementary to SEQ ID No. 6, as well as a composition comprising the oligonucleotide and a physiologically acceptable carrier. Additionally, there is provided a method of treating a neoplastic state in an individual in need of such treatment, comprising the step of (a) administering to the individual an effective dose of the above-described oligonucleotide.

[0020] Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1 shows a comparison of PCR products derived from normal and carcinoma cDNA as shown by staining in an agarose gel. Two distinct bands (lane 2) were present in the primer pair sense-His-antisense Asp (AS1) and multiple bands of about 500 base pairs are noted in the carcinoma lane for the sense-His antisense-Ser (AS2) primer pairs (lane 4).

[0022] FIGS. 2A-2D show the Northern blot analysis of TADG-14. FIG. 2A shows messenger RNA isolated from the tissues of interest was subjected to Northern hybridization using a random labeled 230 bp TADG-14 specific RT-PCR product. The blot was stripped and probed for b-tubulin. FIGS. 2B, 2C, and 2D show multiple tissue Northern blots (Clontech) probed with the same TADG-14 and b-tubulin specific RT-PCR products. TADG-14 mRNA was detected as a 1.4-kb transcript in tumors but not in any normal tissue studied.

[0023] FIGS. 3A-3B shows the cDNA and deduced amino acid sequences of TADG-14 and comparison of predicted TADG-14 sequence with known proteases. FIG. 3A shows the cDNA sequence of TADG-14 with its deduced 260 amino acid sequence represented by the one-letter code for each residue. Within the cDNA, the underlined portions represent the Kozak's consensus sequence for initiation of translation and the polyadenylation signal, respectively. The TADG-14 protein sequence contains a secretion signal sequence near its amino terminus. The stop codon is represented by the (*) symbol. FIG. 3B shows the amino acid sequence of TADG-14 compared to human glandular kallikrein (hHk2, accession # P06870), human PSA (hPSA, accession # P07288), mouse

neuropsin (mNeur, accession # D30785) and human Protease M (hProM, accession # U62801) using the GCG PILEUP program (REF). The positions of the residues of the catalytic triad are marked Y.

[0024] FIG. 4 shows a comparison of the amino acid sequence of TADG-14's catalytic domains.

[0025] FIGS. 5A-5B show the TADG-14 quantitative PCR. FIG. 5A shows the typical results of a TADG-14 quantitative PCR experiment. The reaction products were electrophoresed through a 2% agarose TAE gel and stained with ethidium bromide. In this figure, the 454-bp band represents the b-tubulin product and the 230-bp band represents the TADG-14 product. The radiolabeled PCR products were quantitated. FIG. 5B shows the overexpression of TADG-14. As determined by the student's t test, TADG-14 mRNA expression levels were significantly elevated in LMP tumors (*, $P=0.05$) and carcinomas ($P<0.0001$) compared to levels found in normal ovary. Individual cases are represented in a scatter plot. This is indicative of heterogeneity of TADG-14 expression among these tumor samples.

[0026] FIG. 6 shows the TADG-14 expression in tumors and cell lines.

[0027] FIG. 7 shows Western blot analysis of TADG-14. Polyclonal antibodies were generated by immunization of rabbits with one of three poly-lysine linked multiple antigen peptides derived from the deduced amino acid sequence of TADG-14. For Western blot analysis, approximately 20 ug of MDA-MB-435S and HeLa cell lysates were separated on a 15% SDS-PAGE gel and electroblotted to PVDF at 100V for 40 minutes at 4 C. The blot was blocked overnight in Tris-buffered saline (TBS), pH 7.8 containing 0.2% non-fat milk. Primary antibody was added to the membrane at a dilution of 1:100 in 0.2% milk/TBS and incubated for 2 hours at room temperature. The blot was washed and incubated with 1:3000 dilution of alkaline-phosphatase conjugated goat and anti-rabbit IgG antibody (Bio-Rad) for one hour at room temperature. The blot was washed and incubated with a chemiluminescent substrate (Bio-Rad) before a 10-second exposure to X-ray film for visualization.

[0028] FIGS. 8A-8F show immunohistochemistry of TADG-14. Staining was with the TADG-14-1 antibody for normal ovary, two serous carcinomas, mucinous carcinoma, endometrioid carcinoma and clear cell carcinoma of the ovary (FIG. 8A, FIG. 8B, FIG. 8C, FIG. 8D, FIG. 8E and FIG. 8F, respectively). No staining was observed in normal ovary. The serous carcinoma shown in FIG. 8B has TADG-14 most strongly associated with the surface of the tumor, while in the serous tumor in FIG. 8C, TADG-14 was found in a granular form in an apparent secretion pathway. In mucinous carcinoma TADG-14 appears to be most highly expressed along the invasive front of the tumor. TADG-14 was secreted into the lumen of the glandular structure formed by the endometrioid carcinoma in FIG. 8E. The clear cell carcinoma stained in FIG. 8F shows diffuse staining throughout all tumor cells.

[0029] FIG. 9 shows the expression of TADG-14 and T-14 variant in normal ovary and ovarian carcinoma.

[0030] FIG. 10 presents a diagram of transcript and open reading frame of TADG-14 and TADG-14 variant including intron 2.

[0031] FIG. 11 shows amino acid sequence comparison of TADG-14 and TADG-14 variant.

DETAILED DESCRIPTION OF THE INVENTION

[0032] All serine proteases contain conserved histidine, aspartate and serine residues that are necessary for enzymatic

activity. To identify the expressed serine proteases in carcinoma, degenerate oligodeoxynucleotide primers designed to the conserved amino acid sequences surrounding the invariant His and Ser residues of the catalytic triad (8) were used in PCR reactions with cDNA from either normal ovarian tissue or ovarian carcinoma as the template. PCR products of the appropriate size were subcloned into T-vector and sequenced. Previously, this strategy has proved successful in identifying the serine proteases hepsin and stratum corneum chymotryptic enzyme (SCCE) which have been shown to be expressed at abnormally high levels in ovarian carcinoma (9, 10).

[0033] Homology searches revealed that one of the subclones obtained from ovarian carcinoma represented a novel 406 base pair (bp) sequence that has significant sequence similarity to other known proteases including mouse neuropilin, human glandular kallikrein and human PSA. The complete cDNA for this novel sequence was cloned and found to encode a trypsin like serine protease, named TADG-14. The TADG-14 cDNA is 1343 base pairs long (SEQ ID No: 6) and encoding for a 260 amino acid protein (SEQ ID No: 7).

[0034] The availability of the TADG-14 gene opens the way for a number of studies that can lead to various applications. More importantly, the TADG-14 transcript was found to be highly expressed in a majority of ovarian tumors but not expressed by normal ovarian tissue. High level expression of TADG-14 appears to be restricted to tumors, and this protease appears to be secreted in a manner that would suggest a possible role in invasion and metastasis. Moreover, due to the extracellular nature of this enzyme, it may be possible to exploit its expression as a diagnostic tool for ovarian cancer.

[0035] The present invention also discloses a TADG-14 variant that includes intron sequence between exon 2 and exon 3. This TADG-14 variant could be translated into an extended amino acid sequence which presumably would still have protease activity (FIGS. 10-11). The addition of the intron sequence and the subsequent translation into additional amino acid sequence provides an opportunity to add unique specificity for diagnostic detection and/or targeting of tumor therapy. This variant was expressed in 5 out of 6 ovarian carcinomas examined. Normal ovary cells did not express this TADG-14 variant.

[0036] 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).

[0037] The amino acids 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 is retained by the polypeptide. 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.

[0038] 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 codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0039] 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.

[0040] A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

[0041] 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-14 protein of the present invention can be used to transform a host using any of the techniques commonly known to those of ordinary skill in the art. Especially preferred is the use of a vector containing coding sequences for the gene which encodes a human TADG-14 protein of the present invention for purposes of prokaryote transformation.

[0042] 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.

[0043] The invention includes a substantially pure DNA encoding a TADG-14 protein, 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 1343 of the nucleotides listed in SEQ ID NO: 6. 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: 7. More preferably, the DNA includes the coding sequence of the nucleotides of SEQ ID NO: 6, or a degenerate variant of such a sequence.

[0044] "Substantially pure DNA" is DNA that is part of a milieu in which the DNA does not naturally occurs. The DNA can be obtained by virtue of separation (partial or total purification) of some or all of the molecules of that milieu, or by

virtue of alteration of sequences that flank the claimed DNA. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, an autonomously replicating plasmid or virus, the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by polymerase chain reaction (PCR) or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence, e.g., a fusion protein. Also included is a recombinant DNA which includes a portion of the nucleotides listed in SEQ ID NO: 6 which encodes an alternative splice variant of TADG-14.

[0045] The present invention encompasses DNA that have at least about 70% sequence identity to the coding sequence of the nucleotides listed in SEQ ID NO: 6, preferably at least 75% (e.g. at least 80%); and most preferably at least 90%. The identity between two sequences is a direct function of the number of matching or identical positions. When a 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, 1710 University Avenue, Madison, W 53705).

[0046] The invention also includes DNA that hybridizes at high stringency to a probe containing at least 15 consecutive nucleotides of SEQ ID NO: 6. The probe to which the DNA of the invention hybridizes preferably consists of a sequence of at least 20 consecutive nucleotides, more preferably 40 nucleotides, even more preferably 50 nucleotides, and most preferably 100 nucleotides or more (up to 100%) of the coding sequence of the nucleotides listed in SEQ ID NO: 6 or the complement thereof. Such a probe is useful for detecting expression of TADG-14 in a 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.

[0047] By "high stringency" is meant DNA hybridization and wash conditions characterized by high temperature and low salt concentration, e.g., wash conditions of 65° C. at a salt concentration of approximately 0.1×SSC, or the functional equivalent thereof. For example, high stringency conditions may include hybridization at about 42° C. in the presence of about 50% formamide; a first wash at about 65° C. with about 2×SSC containing 1% SDS; followed by a second wash at about 65° C. with about 0.1×SSC.

[0048] The present invention further comprises a vector comprising a DNA sequence which encodes a human TADG-14 protein or a human TADG-14 variant protein. The vector comprises in operable linkage: a) an origin of replication; b) a promoter; and c) 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: 6 or encode a TADG-14 variant having the amino acid sequence of SEQ ID NO. 75.

[0049] 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 nucleic acid encoding TADG-14 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 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.

[0050] 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 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.

[0051] Further included in this invention are substantially pure TADG-14 protein or TADG-14 variant protein which are encoded at least in part by portions of SEQ ID NO. 7 and SEQ ID NO. 75 respectively. The protein products include alternative mRNA splicing or alternative protein processing events, or in which a section of TADG-14 sequence has been deleted. The fragment, or the intact TADG-14 polypeptide, may be covalently linked to another polypeptide, e.g. which acts as a label, a ligand or a means to increase antigenicity.

[0052] 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-14 protein or TADG-14 variant may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a TADG-14 or TADG-14 variant 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-14 or TADG-14 variant, polyacrylamide gel electrophoresis, or HPLC analysis. A protein which is

chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be, by definition, substantially free from its naturally associated components. Accordingly, substantially pure proteins include eukaryotic proteins synthesized in *E. coli*, other prokaryotes, or any other organism in which they do not naturally occur.

[0053] In addition to substantially full-length proteins, the invention also includes fragments (e.g., antigenic fragments) of the TADG-14 or TADG-14 variant protein. As used herein, "fragment," as applied to a polypeptide, will ordinarily be at least 10 residues, more typically at least 20 residues, and preferably at least 30 (e.g., 50) residues in length, but less than the entire, intact sequence. Fragments of the TADG-14 or TADG-14 variant protein can be generated by methods known to those skilled in the art, e.g., by enzymatic digestion of naturally occurring or recombinant TADG-14 or TADG-14 variant protein, by recombinant DNA techniques using an expression vector that encodes a defined fragment of TADG-14 or TADG-14 variant, or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of TADG-14 (e.g., binding to an antibody specific for TADG-14) can be assessed by methods described herein. Purified TADG-14 or antigenic fragments of TADG-14 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.

[0054] Included in this invention are polyclonal or monoclonal antibodies specific for TADG-14 or TADG-14 variant. 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-14 cDNA clones, and to distinguish them from known cDNA clones.

[0055] The invention encompasses not only an intact polyclonal or monoclonal antibody, but also an immunologically active antibody fragment that recognizes TADG-14 or TADG-14 variant, e.g., a Fab or (Fab)₂ fragment; an engineered single chain Fv molecule; or a chimeric antibody in which the antigen binding sites are derived from murine antibody while the remaining portions of the antibody are of human origin.

[0056] In one embodiment, the antibody, or a fragment thereof, may be linked to a toxin or to a detectable label, e.g. a radioactive label, non-radioactive isotopic label, fluorescent label, chemiluminescent label, paramagnetic label, enzyme label, or colorimetric label. Examples of suitable toxins include diphtheria toxin, *Pseudomonas* exotoxin A, ricin, and cholera toxin. Examples of suitable enzyme labels include malate hydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholinesterase, etc. Examples of suitable radioisotopic labels include ³H, ¹²⁵I, ¹³¹I, ³²P, ³⁵S, ¹⁴C, etc.

[0057] Paramagnetic isotopes for purposes of in vivo diagnosis can also be used according to the methods of this invention. There are numerous examples of elements that are useful in magnetic resonance imaging. For discussions on in vivo nuclear magnetic resonance imaging, see, for example, Schaefer et al., (1989) *JACC* 14, 472-480; Shreve et al., (1986) *Magn. Reson. Med.* 3, 336-340; Wolf, G. L., (1984)

Physiol. Chem. Phys. Med. NMR 16, 93-95; Wesbey et al., (1984) *Physiol. Chem. Phys. Med. NMR* 16, 145-155; Runge et al., (1984) *Invest. Radiol.* 19, 408-415. Examples of suitable fluorescent labels include a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an ophthaldehyde label, a fluorescamine label, etc. Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, an aequorin label, etc.

[0058] 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.

[0059] As described herein, the invention provides a number of diagnostic advantages and uses. For example, the TADG-14 or TADG-14 variant protein is useful in diagnosing cancer in different tissues since this protein is absent in highly proliferating cells. Antibodies (or antigen-binding fragments thereof) which bind to an epitope specific for TADG-14 or TADG-14 variant are useful in a method of detecting TADG-14 protein in a biological sample for diagnosis of cancerous or neoplastic transformation. This method includes the steps of obtaining a biological sample (e.g., cells, blood, plasma, tissue, etc.) from a patient suspected of having cancer, contacting the sample with a labelled antibody (e.g., radioactively tagged antibody) specific for TADG-14 or TADG-14 variant, and detecting the TADG-14 protein using standard immunoassay techniques such as an ELISA. Antibody binding to the biological sample indicates that the sample contains a component which specifically binds to an epitope within TADG-14.

[0060] Likewise, a standard Northern blot assay can be used to ascertain the relative amounts of TADG-14 or TADG-14 variant mRNA in a cell or tissue obtained from a patient suspected of having cancer. Northern assay usually uses a hybridization probe, e.g. a full-length, single stranded radio-labelled TADG-14 cDNA probe having a sequence complementary to SEQ ID NO: 6, or a fragment of that DNA sequence at least 20 (preferably at least 30, more preferably at least 50, and most preferably at least 100) consecutive nucleotides in length. The DNA hybridization probe can be labelled by any of the many different methods known to those skilled in this art.

[0061] In another embodiment of the present invention, there is provided a method of inhibiting expression of TADG-14 in a cell, comprising the step of: (a) introducing a vector into a cell, whereupon expression of the vector produces TADG-14 antisense DNA which hybridizes to endogenous TADG-14 mRNA and inhibits expression of TADG-14 in the cell.

[0062] Further embodied by the present invention, there is provided a method of inhibiting a TADG-14 protein in a cell, comprising the step of: (a) introducing an antibody specific for TADG-14 protein or a fragment thereof into a cell, where-

upon binding of the antibody to the TADG-14 protein inhibits the TADG-14 protein in said cell.

[0063] The present invention is also directed toward a method of targeted therapy to an individual, comprising the step of: (a) administering a compound having a targeting moiety and a therapeutic moiety to an individual, wherein the targeting moiety is specific for TADG-14. Representative targeting moieties are an antibody specific for TADG-14 and a ligand or ligand binding domain that binds TADG-14. Likewise, a representative therapeutic moiety is a radioisotope, a toxin, a chemotherapeutic agent and immune stimulants. Typically, the above-described method is useful when the individual suffers from ovarian cancer, breast cancer or cancers of the prostate, lung, colon and cervix.

[0064] The present invention also provides methods of diagnosing cancer in an individual, comprising the steps of: (a) obtaining a biological sample from an individual; and (b) detecting TADG-14 or TADG-14 variant in the sample. Generally, the presence of TADG-14 or TADG-14 variant in the sample is indicative of the presence of carcinoma in the individual. Generally, the biological sample is blood, ascites fluid, interstitial fluid, tumor tissue biopsy or tumor cells. Typical means of detecting TADG-14 or TADG-14 variant are by Northern blot, Western blot, PCR, dot blot, ELISA, radioimmunoassay, DNA chips or tumor cell labeling. This method may be useful in diagnosing cancers such as ovarian, breast, prostate and colon cancers.

[0065] In still yet another embodiment of the present invention, there is provided a method of vaccinating an individual against TADG-14 protein, comprising the step of (a) inoculating an individual with a TADG-14 protein or fragment thereof which lacks TADG-14 protease activity. Typically, inoculation with the TADG-14 protein or fragment thereof elicits an immune response in the individual, thereby vaccinating the individual against TADG-14. Generally, the individual has cancer, is suspected of having cancer or is at risk of getting cancer. Preferably, the TADG-14 fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 17, 18, 41, 42, 47, 48, 53, 56, or 64.

[0066] In another embodiment of the present invention, there is provided a method of producing activated immune cells directed toward TADG-14, comprising the steps of exposing immune cells to a TADG-14 protein or fragment thereof which lacks TADG-14 protease activity. Usually, exposure to the TADG-14 protein or fragment thereof activates the immune cells, thereby producing activated immune cells directed toward TADG-14. Generally, the immune cells are B cells, T cells or dendritic cells. Preferably, the dendritic cells are isolated from an individual prior to exposure to a TADG-14 protein or fragment thereof, and then reintroduced into the individual subsequent to the exposure. Typically, the individual has cancer, is suspected of having cancer or is at risk of getting cancer. Preferably, the TADG-14 fragment is a 9-residue-fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 17, 18, 41, 42, 47, 48, 53, 56, or 64.

[0067] In another embodiment of the present invention, there is provided an immunogenic composition, comprising an immunogenic fragment of a TADG-14 protein and an appropriate adjuvant. Preferably, the TADG-14 fragment is a 9-residue fragment up to a 20-residue fragment, and more preferably, the 9-residue fragment is SEQ ID Nos. 17, 18, 41, 42, 47, 48, 53, 56, or 64.

[0068] In another embodiment of the present invention, there is provided an oligonucleotide having a sequence complementary to SEQ ID No. 6, as well as a composition comprising the oligonucleotide and a physiologically acceptable carrier. Additionally, there is provided a method of treating a neoplastic state in an individual in need of such treatment, comprising the step of (a) administering to the individual an effective dose of the above-described oligonucleotide.

[0069] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

EXAMPLE 1

Cloning and Characterization of TADG-14

Tissue Collection and Storage

[0070] Upon patient hysterectomy, bilateral salpingo-oophorectomy, or surgical removal of neoplastic tissue, the specimen was retrieved and placed it 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 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 Cooperative Human Tissue Network and shipped on dry ice. Upon arrival, these specimens were logged into the laboratory record and stored at -80°C .

mRNA Isolation and cDNA Synthesis

[0071] Messenger RNA (mRNA) isolation was performed according to the manufacturer's instructions using the Mini RiboSepTM Ultra mRNA isolation kit purchased from Becton Dickinson. This was an oligo(dT) chromatography based system of mRNA isolation. The amount of mRNA recovered was quantitated by UV spectrophotometry.

[0072] First strand complementary DNA (cDNA) was synthesized using 5.0 ug of mRNA and either random hexamer or oligo(dT) primers according to the manufacturer's protocol utilizing a first strand synthesis kit obtained from Clontech. The purity of the cDNA was evaluated by PCR using primers specific for the p53 gene. These primers span an intron such that pure cDNA can be distinguished from cDNA that is contaminated with genomic DNA.

PCR Reactions

[0073] Reactions with degenerate primers and quantitative PCR reactions were carried out as previously described (10, 11). The sequences of the TADG-14 specific primers that produce the 230 bp product were as follows: 5'-ACAG-TACGCCTGGGAGACCA-3' (SEQ ID No. 12) and 5'-CT-GAGACGGTGC AATTCTGG-3' (SEQ ID No. 13).

T-Vector Ligation and Transformations

[0074] The purified PCR products were ligated into the Promega T-vector plasmid and the ligation products were

used to transform JM109 competent cells according to the manufacturer's instructions. Positive colonies were cultured for amplification, the plasmid DNA isolated by means of the Wizard™ Minipreps DNA purification system, and the plasmids were digested with *Apal* and *SacI* restriction enzymes to determine the size of the insert. Plasmids with inserts of the size(s) visualized by the previously described PCR product gel electrophoresis were sequenced.

DNA Sequencing

[0075] Utilizing a plasmid specific primer near the cloning site, sequencing reactions were carried out using PRISM™ Ready Reaction Dye Deoxy™ terminators (Applied Biosystems) according to the manufacturer's instructions. Residual dye terminators were removed from the completed sequencing reaction using a Centri-sep™ spin column (Princeton Separation). An Applied Biosystems Model 373A DNA Sequencing System was used for sequence analysis. Sequences were compared to GenEMBL databases using the FASTA program (Wisconsin Package Version 9.1, Genetics Computer Group (GCG), Madison, Wis.) Multiple sequence alignments were generated with the Bestfit and Pileup programs available through Genetics Computer Group.

Northern Blot Analysis

[0076] mRNAs (approximately 5 mg) were size separated by electrophoresis through a 6.3% formaldehyde, 1.2% agarose gel in 0.02 M MOPS, 0.05 M sodium acetate (pH 7.0), and 0.001 M EDTA. The mRNAs were then blotted to Hybond-N (Amersham) by capillary action in 20×SSPE. The RNAs were fixed to the membrane by baking for 2 hours at 80° C. Additional multiple tissue northern (MTN) blots were purchased from CLONTECH Laboratories, Inc., including the Human multiple tissue northern blot (cat. #7760-1), the Human multiple tissue northern II blot (cat. #7759-1), the Human Fetal multiple tissue northern II blot (cat. #7756-1), and the Human Brain multiple tissue northern III blot (cat. #7750-1). The 230 bp TADG-14 specific PCR product was radiolabelled utilizing the Prime-a-Gene Labelling System available from Promega. The blots were probed and stripped according to the ExpressHyb Hybridization Solution protocol available from CLONTECH.

Antibody Production and Western Blot Analysis

[0077] Polyclonal antibodies were generated by immunization of white New Zealand rabbits with one of three polylysine linked multiple antigen peptides derived from the deduced amino acid sequence of TADG-14. These sequences are KYTVRLGDHSLQ (T14-1, SEQ ID No. 14), GHECQPHSQPWQ (T14-2, SEQ ID No. 15), and LDWIKKIIIGSKG (T14-3, SEQ ID No. 16). For Western blot analysis, approximately 20 µg of MDA-MB-435S and HeLa cell lysates were separated on a 15% SDS-PAGE gel and electrophoretically transferred to PVDF at 100V for 40 minutes at 4° C. The blot was blocked overnight in Tris-buffered saline (TBS), pH 7.8 containing 0.2% non-fat milk. Primary antibody was added to the membrane at a dilution of 1:100 in 0.2% milk/TBS and incubated for 2 hours at room temperature. The blot was washed and incubated with a 1:3000 dilution of alkaline-phosphatase conjugated goat anti-rabbit IgG antibody (Bio-Rad) for one hour at room temperature. The blot was washed

and incubated with a chemiluminescent substrate (Bio-Rad) before a 10-second exposure to X-ray film for visualization.

Immunohistochemistry

[0078] Immunohistochemical staining was performed using a 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 in methanol with 0.3% H₂O₂ for 30 minutes at room temperature and then incubated with normal goat serum for 30 minutes. The samples were incubated with anti-TADG-14 peptide derived polyclonal antibody for 1 hour at room temperature in a moisture chamber, followed by incubation with biotinylated anti-rabbit IgG for 30 minutes, and then incubated with ABC reagent (Vector) for 30 minutes. The final products were visualized using the AEC substrate system (DAKO) and sections were counter stained with hematoxylin before mounting. Negative controls were performed by using normal serum instead of the primary antibody.

Cloning Results

[0079] The gene encoding the novel extracellular serine protease of the present invention was identified from a group of proteases overexpressed in carcinoma by subcloning and sequencing the appropriate PCR products. An example of such a PCR reaction is given in FIG. 1. Subcloning and sequencing of individual bands from such an amplification provided a basis for identifying the novel protease of the present invention.

[0080] After confirming the 406 bp PCR product was unique and was appropriately conserved to fit into the serine protease family, this PCR product was used as a probe for Northern blot analysis to determine the transcript size and tissue specificity of its expression. It was found that the mRNA for this clone is approximately 1.4 kilobases (kb) (FIG. 2A), and that it is strongly expressed in ovarian carcinomas but not in normal ovary. More importantly, the transcript was found to be undetectable by Northern analysis in 28 normal human tissues studied (FIGS. 2B, 2C, 2D and data not shown). In a more sensitive assay of 50 normal human tissues (Clontech), RNA dot blot analysis revealed that this clone was very weakly expressed in only three of these 50 tissues, kidney, lung and mammary gland (data not shown).

[0081] Using standard hybridization techniques, a cDNA library constructed from the mRNA isolated from the ascites cells of an ovarian cystadenocarcinoma patient was screened. Five clones were obtained, two of which overlapped and spanned 1343 nucleotides (FIG. 3A). The last two nucleotides prior to the poly (A) tail and the poly (A) tail itself were obtained from the EST database available at NCBI (accession # AA343629). Subsequent Northern blot analyses with probes derived from sequences near the 5' or 3' end of this cDNA were consistent with previous results suggesting that the obtained clones were produced by the same gene (data not shown). This cDNA includes a Kozak's consensus sequence for the initiation of translation, and a polyadenylation signal.

[0082] The mRNA provides an open reading frame of 260 amino acids, which contains the necessary residues (His⁷³, Asp¹²⁰, Ser²¹²) in the appropriate context to classify this protein as a trypsin-like serine protease (1). Near its amino-terminus, the predicted protein contains a stretch of hydrophobic amino acids that probably serve as a secretion signal

sequence. In addition, residues 110 to 112 encode a potential site for glycosylation that is common to serine proteases of the kallikrein subfamily such as PSA. This enzyme was named TADG-14, and the sequence was submitted to GenBank and assigned the accession # AF055982.

[0083] Comparison of the deduced TADG-14 amino acid sequence with sequences of known proteases revealed that it possesses significant similarity with human glandular kallikrein (hHk2), PSA, Protease M and mouse neuropsin (11-14). The sequence determined for the catalytic domain of TADG-14 is presented in FIG. 4 and is consistent with other serine proteases and specifically contains conserved amino acids appropriate for the catalytic domain of the serine protease family. At the amino acid level TADG-14 is 48% identical to Protease M, 46% identical to hHk2, and 43% identical to PSA (FIG. 3B). More interestingly, the mouse protease neuropsin and TADG-14 share 72% amino acid identity. In addition to the similarity of the protein sequences, neuropsin and TADG-14 mRNAs are of similar size (1.4 kb) and structure with approximately the same amounts of 5' and 3' untranslated regions suggesting the possibility of orthology. Neuropsin was originally identified as being expressed in mouse hippocampus and shown to be differentially expressed under stimulation (14). However, TADG-14 mRNA was undetectable in human whole brain by Northern blot. Further, Northern blot analysis for TADG-14 in eight separate parts of human brain including amygdala, caudate nucleus, corpus callosum, hippocampus, whole brain, substantia nigra, subthalamic nucleus and thalamus, also turned out to be negative. Recently, a human cDNA encoding neuropsin has been submitted to the GenBank database (accession # AB009849). Although this clone represents a different transcript from TADG-14, it encodes a protein that is identical to TADG-14 (15). Therefore, it seems logical that TADG-14 and neuropsin may arise as alternative splicing products from the same gene.

[0084] To characterize the extent and frequency of expression of the TADG-14 gene in ovarian tumors, semi-quantitative PCR was performed with cDNA derived from normal ovary, ovarian carcinoma or low malignant potential (LMP) tumors as template. This technique has been previously authenticated and verified by Northern blot, Western blot and immunohistochemistry (9, 16). PCR primers that amplify a TADG-14 specific 230 bp product were synthesized and used simultaneously in reactions with primers that produce a specific 454 bp PCR product for β -tubulin. A radiolabelled nucleotide was included in this reaction, the PCR products were separated on a 2% agarose gel and the intensity of each band was quantitated by a Phosphorimager (Molecular Dynamics). FIG. 5A shows an ethidium bromide stained agarose gel with the separated quantitative PCR products and is representative of the typical results observed.

[0085] The ratio of the TADG-14 PCR product to that of β -tubulin (mean $\pm$ SD) was calculated for normal ovary samples which all showed relatively low expression levels (0.034 $\pm$ 0.024). TADG-14 overexpression was defined as exceeding the mean of the ratio of TADG-14 to β -tubulin for normal samples by greater than 2 standard deviations (SD). TADG-14 was found to be overexpressed in 4 of 10 LMP tumors (40%), and 20 of 30 ovarian carcinomas (67%) studied. For individual histologic subtypes of tumor, the expression ratio was 0.110 $\pm$ 0.092 for serous LMP tumors, 0.096 $\pm$ 0.142 for mucinous LMP tumors, 0.457 $\pm$ 0.345 for serous carcinomas, 0.171 $\pm$ 0.300 for mucinous carcinomas, 0.308 $\pm$ 0.144 for clear cell carcinomas, and 0.485 $\pm$ 0.325 for

endometrioid carcinomas. Of the 30 ovarian carcinomas studied, 13 of 17 serous tumors, 1 of 7 mucinous tumors, 3 of 3 clear cell tumors and 3 of 3 endometrioid tumors overexpressed TADG-14 (FIG. 5B). These data are summarized in Table 1. Although not quantitated, transcripts for TADG-14 were also detectable in breast and colon carcinoma (FIG. 6).

TABLE 1

TADG-14 Overexpression by Tissue Subtype	
Tissue Type	TADG14 Overexpression
Normal	0/10 (0%)
LMP	4/10 (40%)
Serous	3/6 (50%)
MuCinous	1/4 (25%)
Carcinoma	20/30 (67%)
Serous	13/17 (76%)
MuCinous	1/7 (14%)
Endometrioid	3/3 (100%)
Clear Cell	3/3 (100%)

[0086] Immunogenic poly-lysine linked multiple antigen peptides were synthesized based on the deduced amino acid sequence of TADG-14 and used to immunize rabbits for the production of polyclonal antibodies. The antiserum raised to the peptide sequence LDWIKKIIGSKG (SEQ ID No. 16), near the carboxy terminal (AA #249-260), was used in Western blot analysis to determine if this antibody would recognize a protein of the predicted size of 28 kDa. Proteins from the HeLa cell line and the carcinoma derived MD-MBA-435S cell line were used in this experiment and it was found that the antibody recognized a single 30 kDa protein in both cell lines (FIG. 7, lanes 3 and 4). This size is within a reasonable range of the predicted molecular weight. As a negative control, duplicate HeLa and MD-MB435S lysates were examined with rabbit pre-immune serum (FIG. 7, lanes 1 and 2). More importantly, this experiment was reproducible with antisera to a peptide from a different region of TADG-14, suggesting that these cultured cancer cells produce the TADG-14 protein.

[0087] Immunohistochemical staining supported the data obtained by quantitative PCR and Northern blot. Using a TADG-14 peptide directed antibody, no staining was observed with normal ovarian tissue samples (FIG. 8A). However, intense staining was associated with tumor cells of all of the various histological subtypes of ovarian carcinoma examined. For serous carcinoma (FIGS. 8B and 8C) the antigen appears to be associated with tumor cells in the form of granules. These granular structures may be intermediates in the pathway that ultimately leads to secretion of TADG-14. In mucinous and clear cell carcinoma samples (FIGS. 8D and 8F respectively), TADG-14 is highly associated with the tumor cells. In endometrioid carcinoma (FIG. 8E), the antigen is most prevalent in the glandular lumen formed by the tumor cells.

[0088] The lethality of neoplastic cells lies in their ability to proliferate abnormally and invade normal host tissues. Malignancies employ proteases to provide a variety of services that assist in the process of tumor progression including activation of growth and angiogenic factors and to provide the basis for invasion and metastasis. In the process of studying these enzymes, overexpression of known proteases such as hepsin and SCCE have been identified. In the present study, a cDNA encoding a novel serine protease, TADG14, was cloned. This protease was found to be very highly expressed in 67% (20/30) of ovarian carcinomas studied, whereas it was undetected

in normal ovarian tissue. The TADG14 transcript was also not detectable in any of 50 normal human tissues studied. On prolonged Northern blot exposure, extremely low levels of TADG-14 were detected in normal kidney, breast and lung. This suggests the possibility that this gene is under the control of a promoter that is most active in ovarian tumors, and it may be possible to exploit this for therapeutic means. Unfortunately, TADG14 expression can be detected in other types of cancer including prostate, breast and colon. This may limit the usefulness of TADG14 as a potential diagnostic maker for ovarian carcinoma, but it in no way detracts from the usefulness of this molecule as a target for cancer therapy or the usefulness of the TADG14 promoter in gene therapy applications.

[0089] At the nucleotide level, TADG14 mRNA resembles the recently cloned human neuropsin transcript with obvious differences residing in the 5' and 3' UTRS. TADG14 mRNA contains 491 bases of 5' UTR that were not found in human neuropsin. Also, the nucleotides preceding the poly (A) tail in the 3' UTR are not homologous. A 0.9 kb transcript for human neuropsin was identified in cultured keratinocytes but not in normal hippocampus. Also, it was not identified as being associated with tumors. At the amino acid level, TADG14 is identical to human neuropsin.

[0090] Among other known proteases, TADG14 most closely resembles the mouse protease known as neuropsin, which was originally cloned from mouse hippocampus, and subsequently implicated in neuronal plasticity (17). If TADG14 functions in a manner similar to mouse neuropsin, it may be capable of restructuring the three-dimensional architecture of a tumor allowing for shedding of tumor cells or invasion of normal host tissues by degrading fibronectin (18). In support of this, immunohistochemical staining of ovarian tumors revealed that TADG14 is highly associated with tumor cells and the cells near the invasive fronts of tumor. Therefore, TADG14 could be an important target for the inhibition of tumor progression.

[0091] Most importantly, the five-year survival rate for ovarian cancer patients remains below 50% because of an inability to diagnose this disease at an early stage. TADG14 contains a secretion signal sequence and immunohistochemical data suggest that TADG14 is secreted. In addition, by Northern blot and RNA dot blot analyses, TADG14 appears only in abundance in tumor tissues. As a result of this, it may be possible to design assays based on the detection of this protein for the early detection of ovarian cancer. Currently, the best available ovarian cancer tumor marker is CA 125. However, due to high endogenous circulating levels of this antigen, the signal to noise ratio limits its usefulness as a diagnostic tool. Therefore, TADG14, due to its limited expression in other tissues and potential for being present in the circulation of tumor bearing patients, may prove to be a useful tool for early detection of ovarian cancer, especially the most prevalent serous cystadenocarcinoma subtype.

EXAMPLE 2

Peptide Ranking Analysis for Vaccine Candidates

[0092] For vaccine or immune stimulation, individual 9-mers to 11-mers of the TADG-14 protein were examined to rank the binding of individual peptides to the top 8 haplotypes in the general population (Parker et al., (1994)). Table 2 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-14 peptides that strongly bind to an HLA allele are putative immunogens, and are used to inoculate an individual against TADG-14.

TABLE 2

TADG-14 Peptide Ranking				
HLA Type & Ranking	Start	Peptide	Predicted Dissociation _{1/2}	SEQ ID No.
HLA A0201				
1	55	QLLCGGVLV	257.342	17
2	15	LLLLGGAWA	171.868	18
3	60	GVLVGGNWV	123.846	19
4	61	VLVGGNWVL	111.672	20
5	49	ALFQGGQQL	79.041	21
6	10	KTWMLLLL	75.331	22
7	131	SLGSKVKPI	23.995	23
8	122	MLLQLRDQA	15.312	24
9	124	LQLRDQASL	13.624	25
10	170	TLNCAEVKI	10.433	26
HLA A0205				
1	124	LQLRDQASL	28.560	25
2	10	KTWMLLLL	25.200	22
3	49	ALFQGGQQL	21.000	21
4	208	CQGDSCGGL	16.800	27
5	54	QLLCGGVL	16.800	28
6	61	VLVGGNWVL	14.280	20
7	60	GVLVGGNWV	12.000	19
8	62	LVGGNWVLT	6.800	29
9	55	QLLCGGVLV	6.000	17
10	191	QITDGMVCA	3.000	30
HLA A1				
1	173	CAEVKIFPQ	4.500	31
2	83	LGDHSLQNK	2.500	32
3	183	KCEDAYPGQ	1.800	33
4	192	ITDGMVCAG	1.250	34
5	71	AAHCKKPKY	1.000	35
6	113	DVEDHNHDL	0.900	36
7	229	GSDPCGRSD	0.750	37
8	111	SSDVEDHNH	0.750	38
9	28	AQEDKVLGG	0.675	39
10	217	VCDGALQGI	0.500	40
HLA A24				
1	241	VYTNICRYL	280.000	41
2	247	RYLDWIKKI	198.000	42
3	10	KTWMLLLL	8.000	22
4	7	RAAKTWMFL	8.000	43
5	42	HSQPWQAAL	7.200	44
6	48	AALFQGGQL	7.200	45
7	113	DVEDHNHDL	7.200	36
8	54	QLLCGGVL	6.000	28
9	214	GPLVCDGAL	6.000	46
10	61	VLVGGNWVL	6.000	20
HLA B7				
1	80	TVRLGDHSL	200.000	47
2	5	RPRAAKTWM	200.000	48
3	214	GPLVCDGAL	80.000	46
4	48	AALFQGGQL	36.000	45
5	8	AAKTWMFL	36.000	49
6	3	RPRPRAKT	20.000	50
7	162	SPRENFPDT	20.000	51
8	188	YPGQITDGM	20.000	52
9	7	RAAKTWMFL	12.000	43
10	49	ALFQGGQQL	12.000	21

TABLE 2-continued

TADG-14 Peptide Ranking				
HLA Type & Ranking	Start	Peptide	Predicted Dissociation _{1/2}	SEQ ID No.
HLA B8				
1	133	GSKVKPISL	80.000	53
2	8	AAKTWMFLL	16.000	49
3	3	RPRPRAAKT	8.000	50
4	80	TVRLGDHSL	4.000	47
5	73	HCKKPKYTV	2.400	54
6	131	SLGSKVKPI	2.000	23
7	5	RPRAAKTWM	2.000	48
8	162	SPRENFDPDT	1.200	51
9	214	GPLVCDGAL	0.800	46
10	179	FPQKKCEDA	0.800	55
HLA B2702				
1	234	GRSDKPGVY	200.000	56
2	246	CRYLDWIKK	20.000	57
3	101	VQSIPHPCY	20.000	58
4	43	SQPWQAALF	20.000	59
5	6	PRAAKTWMF	20.000	60
6	26	SRAQEDKVL	18.000	61
7	126	LRDQASLGS	10.000	62
8	149	GQKCTVSGW	10.000	63
9	124	LQLRDQASL	6.000	25
10	54	QQLLCGGVL	6.000	28
HLA B4403				
1	96	QEIPVVQSI	180.000	64
2	71	AAHCKKPKY	13.500	35
3	171	LNCAEVKIF	4.500	65
4	184	CEDAYPGQI	4.000	66
5	114	VEDHNHDLN	4.000	67
6	101	VQSIPHPCY	2.250	68
7	236	SDKPGVYTN	1.800	69
8	164	RENFPDTLN	1.800	70
9	174	AEVKIFPQK	1.600	71
10	43	SQPWQAALF	1.500	59

EXAMPLE 3

TADG-14 Variant

[0093] Because members of the serine protease family are highly expressed and secreted by tumors, they offer potential targets for both diagnosis and therapy. While many of these enzymes are predominantly tumor produced, there is often some level of expression in a limited number of normal tissues. To further enhance the potential for more specific tumor diagnosis and targeting, it would be helpful to provide unique sequences which might be included in the enzyme families. The present example discloses a transcription variant of TADG-14. The TADG-14 variant includes unique intron sequence which could provide potential specificity to the recognition of TADG-14 in tumor.

[0094] Data presented above indicated TADG-14 overexpression in ovarian tumors. Kishi et al. confirmed the overexpression of KLK8 (Neuropsin/Ovasin), which was described as TADG-14 here, as highly overexpressed in the ascites fluid of ovarian cancer patients and presented at elevated levels in serum of 62% of ovarian cancer patients.

[0095] When the complete transcript of the TADG-14 gene was examined for potential variants, one variant was detected which included intron sequence between exon 2 and exon 3. Using primers from exon 1 and exon 3 of TADG-14, a PCR

band larger than the expected exon 2-exon 3 band was detected in ovarian tumor cells (FIG. 9). The sequence of this TADG-14 variant was confirmed. It included all intron 2 sequence and it could be translated into an extended amino acid sequence which presumably would still have protease activity (FIGS. 10-11). The addition of the intron sequence and the subsequent translation into additional amino acid sequence provides an opportunity to add unique specificity for diagnostic detection and/or targeting of tumor therapy. This variant was expressed in 5 out of 6 ovarian carcinomas examined. Normal ovary cells did not express this TADG-14 variant. On examination of the EST database for taq sequence for intron 2 of TADG-14, only one expressed sequence was detected in an adenocarcinoma cell line of the ovary. These data thus suggest a potential role for this TADG-14 variant in both detecting and targeting of ovarian carcinomas.

[0096] Sense and antisense primers were made to exons 2 and 3 of TADG-14 (TADG-14 Sense, 5'-CGA CCT CGT GCG GCC AAG ACG-3', SEQ ID NO. 73; TADG-14 Antisense, 5'-CAG CTG TAA GGA CCC AGT TGC-3', SEQ ID NO. 74). All PCR was run in 20 ul reactions consisting of ovarian tumor cDNA derived from 50 ng of mRNA, 5 pmol each of sense and antisense primers, 0.2 mmol of dNTPs, 2.5 mmol of MgCl₂ and 1U of Taq polymerase in 1x buffer. This mixture was subjected to 1.5 minutes of denaturation at 94° C. followed by 35 cycles of PCR consisting of the following: denaturation for 30 seconds at 94° C., 30 seconds of annealing at the appropriate temperature for each primer set, and 1 minute of extension at 72° C. with an additional 7 minutes of extension on the last cycle.

[0097] The following references were cited herein:

- [0098] 1. Neurath, H. The Diversity of Proteolytic Enzymes. In: R. J. Beynon and J. S. Bond (eds.), pp. 1-13, Proteolytic enzymes, Oxford: IRL Press, 1989
- [0099] 2. Liotta et al., Cell, 64: 327-336, 1991.
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- [0102] 5. Monsky et al., Cancer Res., 53: 3159-3164, 1993.
- [0103] 6. Powell et al., Cancer Res., 53: 417-422, 1993.
- [0104] 7. McCormack et al., Urology, 45: 729-744, 1995.
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- [0108] 11. Evans et al., Biochemistry, 27: 3124-3129, 1988.
- [0109] 12. Watt et al., Proc. Natl. Acad. Sci. U.S.A., 83: 3166-3120, 1986.
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- [0111] 14. Chen et al., J. Neuroscience, 15: 5088-5097, 1995.
- [0112] 15. Yoshida et al., Gene 213: 9-16, 1998.
- [0113] 16. Shigemasa et al., J. Soc. Gynecol. Invest. 4: 95-102, 1997.
- [0114] 17. Suzuki et al., Neuroscience Res., 23: 345-351, 1995.
- [0115] 18. Shimizu et al., J Biol Chem, 273: 11189-11196, 1998.
- [0116] Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

 SEQUENCE LISTING

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Gln Ser Ser Val Val Arg Ala Val Ile His Pro Asp Tyr Asp Ala
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Ala Ser His Asp Gln Asp Ile Met Leu Leu Arg Leu Ala Arg Pro
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Ala Lys Leu Ser Glu Leu Ile Gln Pro Leu Pro Leu Glu Arg Asp
          65           70           75

Cys Ser Ala Asn Thr Thr Ser Cys His Ile Leu Gly Trp Gly Lys
          80           85           90

Thr Ala Asp Gly Asp Phe Pro Asp Thr Ile Gln Cys Ala Tyr Ile
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His Leu Val Ser Arg Glu Glu Cys Glu His Ala Tyr Pro Gly Gln
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          35           40           45

Ser Asp Val Glu Asp His Asn His Asp Leu Met Leu Leu Gln Leu
          50           55           60

Arg Asp Gln Ala Ser Leu Gly Ser Lys Val Lys Pro Ile Ser Leu
          65           70           75

Ala Asp His Cys Thr Gln Pro Gly Gln Asn Cys Thr Val Ser Gly
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35	45
Lys Thr Leu Asn Asn Asp Ile Met Leu Ile Lys Leu Ser Ser Arg	
50	60
Ala Val Ile Asn Ala Arg Val Ser Thr Ile Ser Leu Pro Thr Ala	
65	75
Pro Pro Ala Thr Gly Thr Lys Cys Leu Ile Ser Gly Trp Gly Asn	
80	90
Thr Ala Ser Ser Gly Ala Asp Tyr Pro Asp Glu Leu Gln Cys Leu	
95	105
Asp Ala Pro Val Leu Ser Gln Ala Lys Cys Glu Ala Ser Tyr Pro	
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Lys Leu Ile Ser Pro Gln Asp Cys Thr Lys Val Tyr Lys Asp Leu
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Gly Tyr Leu Pro Phe Arg Asp Pro Asn Ser Glu Glu Asn Ser Asn
50 55 60

Asp Ile Ala Leu Val His Leu Ser Ser Pro Leu Pro Leu Thr Glu
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Tyr Ile Gln Pro Val Cys Leu Pro Ala Ala Gly Gln Ala Leu Val
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Asp Gly Lys Ile Cys Thr Val Thr Gly Trp Gly Asn Thr Gln Tyr
95 100 105

Tyr Gly Gln Gln Ala Gly Val Leu Gln Glu Ala Arg Val Pro Ile
110 115 120

Ile Ser Asn Asp Val Cys Asn Gly Ala Asp Phe Tyr Gly Asn Gln
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<220> FEATURE:
<223> OTHER INFORMATION: Amino acid sequence of TADG-14 protein

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<400> SEQUENCE: 7

```

```

Met Gly Arg Pro Arg Pro Arg Ala Ala Lys Thr Trp Met Phe Leu
1           5           10          15

```

```

Leu Leu Leu Gly Gly Ala Trp Ala Gly His Ser Arg Ala Gln Glu
20          25          30

```

```

Asp Lys Val Leu Gly Gly His Glu Cys Gln Pro His Ser Gln Pro
35          40          45

```

```

Trp Gln Ala Ala Leu Phe Gln Gly Gln Gln Leu Leu Cys Gly Gly
50          55          60

```

```

Val Leu Val Gly Gly Asn Trp Val Leu Thr Ala Ala His Cys Lys

```

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

<210> SEQ ID NO 8
 <211> LENGTH: 799
 <212> TYPE: DNA
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <223> OTHER INFORMATION: Nucleotide sequence of mouse neuropsin homologous to TADG-14

<400> SEQUENCE: 8

agaggccacc atgggacgcc cccaccctg tgcaatccag ccgtggatcc	50
ttctgcttct gttcatggga gcgtgggcag ggctcaccag agctcagggc	100
tccaagatcc tggaaagtcg agagtgtata cccactccc agccttggca	150
ggcagccttg ttccagggcg agagactgat ctgtgggggt gtcctggttg	200
gagacagatg ggtcctcacg gcagcccact gcaaaaaaca gaagtactcc	250
gtgcgtcttg gtgatcatag cctccagagc agagatcagc cggagcagga	300
gatccagggtg gctcagtcta tccagcatcc ttgctacaac aacagcaacc	350
cagaagatca cagtcacgat ataagtctca ttcgactgca gaactcagca	400
aacctcgggg acaaggtgaa gccgggtccaa ctggccaatc tgtgtccaa	450
agttggccag aagtgcata tatcaggctg gggcactgtc accagccctc	500
aagagaactt tccaaacacc ctcaactgtg cggaagtgaa aatctattcc	550
cagaacaagt gtgagagagc ctatccaggg aagatcaccg agggcatggt	600

-continued

ctgtgctggc agcagcaatg gagctgacac gtgccagggt gactcaggag	650
gccctctggg gtgcgacggg atgctccagg gcatacacctc atggggctca	700
gacccctgtg ggaaacccga gaaacctgga gtctacacca aaatctgccg	750
ctacactacc tggatcaaga agaccatgga caacaggqac tgatcctgg	799

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<210> SEQ ID NO 9
<211> LENGTH: 799
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Nucleotide sequence of TADG-14 homologous to
mouse neuropsin
```

<400> SEQUENCE: 9

agacctcacc	atgggacgcc	cccgacctcg	tgcggccaag	acgtggatgt	50
tctctgtctt	gctgggggga	gcctgggag	gacctccag	ggcacaggag	100
gacaagggtg	tgggggggtc	tgagtgccaa	ccccattcgc	agccttgga	150
ggcggccttg	ttccagggcc	agcaactact	ctgtggcgg	gtcctttag	200
gtggcaactg	ggtccttaca	gctgccact	gtaaaaaac	gaaatacaca	250
gtacgcctgg	gagaccacag	cctacagaat	aaagatggc	cagagcaaga	300
aatacctgtg	gttcagtc	tcccacacc	ctgtctaac	agcagcgatg	350
tggaggacca	caaccatgat	ctgatgcttc	ttcaactg	tgaccaggca	400
tccctgggg	ccaaagtga	gcccatcag	ctggcagatc	attgcacca	450
gcctggccag	aagtgcaccg	tctcaggtg	gggcactgtc	accagtcccc	500
gagagaattt	tctgacact	ctcaactgtg	cagaagtaaa	aatctttccc	550
cagaagaagt	gtgaggatgc	ttaccgggg	cagatcacag	atggcatggt	600
ctgtgcaggc	agcagcaaa	gggctgacac	gtgccagggc	gattctggag	650
gccccctggt	gtgtgatggt	gcactccagg	gcatacatc	ctggggctca	700
gacccctgtg	ggagggtccga	caaacctggc	gtctatacca	acatctgccg	750
ctacctqqac	tqqatcaaqa	agatcataqq	caqcaaaqqc	tqattctag	799

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<210> SEQ ID NO 10
<211> LENGTH: 260
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<223> OTHER INFORMATION: Amino acid sequence of mouse neuropsin
homologous to TADG-14
```

<400> SEQUENCE: 10

Met	Gly	Arg	Pro	Pro	Pro	Cys	Ala	Ile	Gln	Pro	Trp	Ile	Leu	Leu
1				5					10					15
Leu	Leu	Phe	Met	Gly	Ala	Trp	Ala	Gly	Leu	Thr	Arg	Ala	Gln	Gly
			20					25					30	
Ser	Lys	Ile	Leu	Glu	Gly	Arg	Glu	Cys	Ile	Pro	His	Ser	Gln	Pro
		35					40					45		
Trp	Gln	Ala	Ala	Leu	Phe	Gln	Gly	Glu	Arg	Leu	Ile	Cys	Gly	Gly
	50					55					60			
Val	Leu	Val	Gly	Asp	Arg	Trp	Val	Leu	Thr	Ala	Ala	His	Cys	Lys
65					70					75				

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Lys Gln Lys Tyr Ser Val Arg Leu Gly Asp His Ser Leu Gln Ser
 80 85 90
 Arg Asp Gln Pro Glu Gln Glu Ile Gln Val Ala Gln Ser Ile Gln
 95 100 105
 His Pro Cys Tyr Asn Asn Ser Asn Pro Glu Asp His Ser His Asp
 110 115 120
 Ile Met Leu Ile Arg Leu Gln Asn Ser Ala Asn Leu Gly Asp Lys
 125 130 135
 Val Lys Pro Val Gln Leu Ala Asn Leu Cys Pro Lys Val Gly Gln
 140 145 150
 Lys Cys Ile Ile Ser Gly Trp Gly Thr Val Thr Ser Pro Gln Glu
 155 160 165
 Asn Phe Pro Asn Thr Leu Asn Cys Ala Glu Val Lys Ile Tyr Ser
 170 175 180
 Gln Asn Lys Cys Glu Arg Ala Tyr Pro Gly Lys Ile Thr Glu Gly
 185 190 195
 Met Val Cys Ala Gly Ser Ser Asn Gly Ala Asp Thr Cys Gln Gly
 200 205 210
 Asp Ser Gly Gly Pro Leu Val Cys Asp Gly Met Leu Gln Gly Ile
 215 220 225
 Thr Ser Trp Gly Ser Asp Pro Cys Gly Lys Pro Glu Lys Pro Gly
 230 235 240
 Val Tyr Thr Lys Ile Cys Arg Tyr Thr Thr Trp Ile Lys Lys Thr
 245 250 255
 Met Asp Asn Arg Asp
 260

<210> SEQ ID NO 11

<211> LENGTH: 244

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

 <223> OTHER INFORMATION: Amino acid sequence of human protease m (hProM);
 accession no. U62801

<400> SEQUENCE: 11

Met Lys Lys Leu Met Val Val Leu Ser Leu Ile Ala Ala Ala Trp
 1 5 10 15
 Ala Glu Glu Gln Asn Lys Leu Val His Gly Gly Pro Cys Asp Lys
 20 25 30
 Thr Ser His Pro Tyr Gln Ala Ala Leu Thr Tyr Ser Gly His Leu
 35 40 45
 Leu Cys Gly Gly Val Leu Ile His Pro Leu Trp Val Leu Thr Ala
 50 55 60
 Ala His Cys Lys Lys Pro Asn Leu Gln Val Phe Leu Gly Lys His
 65 70 75
 Asn Leu Arg Gly Arg Glu Ser Ser Gln Glu Gln Ser Ser Val Val
 80 85 90
 Arg Ala Val Ile His Pro Asp Tyr Asp Ala Ala Ser His Asp Gln
 95 100 105
 Asp Ile Met Leu Leu Arg Leu Ala Arg Pro Ala Lys Leu Ser Glu
 110 115 120
 Leu Ile Gln Pro Leu Pro Leu Glu Arg Asp Cys Ser Ala Asn Thr

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125	130	135
Thr Ser Cys His Ile Leu Gly Trp Gly Lys Thr Ala Asp Gly Asp		
140	145	150
Phe Pro Asp Thr Ile Gln Cys Ala Tyr Ile His Leu Val Ser Arg		
	155	160
Glu Glu Cys Glu His Ala Tyr Pro Gly Gln Ile Thr Gln Asn Met		
	170	175
Leu Cys Ala Gly Asp Glu Lys Tyr Gly Lys Asp Ser Cys Gln Gly		
	185	190
Asp Ser Gly Gly Pro Leu Val Cys Gly Asp His Ile Arg Gly Leu		
	200	205
Val Ser Trp Gly Asn Ile Pro Cys Gly Ser Lys Glu Lys Pro Gly		
	215	220
Val Tyr Thr Asn Val Cys Arg Tyr Thr Asn Trp Ile Gln Lys Thr		
	230	235

Ile Gln Ala Lys

<210> SEQ ID NO 12
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <221> NAME/KEY: primer
 <223> OTHER INFORMATION: Sense primer for TADG14 specific PCR

<400> SEQUENCE: 12

acagtacgcc tgggagacca

20

<210> SEQ ID NO 13
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <221> NAME/KEY: primer
 <223> OTHER INFORMATION: Anti-sense primer for TADG14 specific PCR

<400> SEQUENCE: 13

ctgagacggt gcaattctgg

20

<210> SEQ ID NO 14
 <211> LENGTH: 12
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Peptide sequence of immunogenic poly-lysine
 linked multiple antigen (T14-1) derived from
 TADG-14 used to produce polyclonal antibodies

<400> SEQUENCE: 14

Lys Tyr Thr Val Arg Leu Gly Asp His Ser Leu Gln
1 5 10

<210> SEQ ID NO 15
 <211> LENGTH: 12
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Peptide sequence of immunogenic poly-lysine
 linked multiple antigen (T14-2) derived from
 TADG-14 used to produce polyclonal antibodies

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<400> SEQUENCE: 15

Gly His Glu Cys Gln Pro His Ser Gln Pro Trp Gln
1 5 10

<210> SEQ ID NO 16

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Peptide sequence of immunogenic poly-lysine
linked multiple antigen (T14-3) derived from
TADG-14 used to produce polyclonal antibodies

<400> SEQUENCE: 16

Leu Asp Trp Ile Lys Lys Ile Ile Gly Ser Lys Gly
1 5 10

<210> SEQ ID NO 17

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 55-63 of the TADG-14 protein

<400> SEQUENCE: 17

Gln Leu Leu Cys Gly Gly Val Leu Val
1 5

<210> SEQ ID NO 18

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 15-23 of the TADG-14 protein

<400> SEQUENCE: 18

Leu Leu Leu Leu Gly Gly Ala Trp Ala
1 5

<210> SEQ ID NO 19

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 60-68 of the TADG-14 protein

<400> SEQUENCE: 19

Gly Val Leu Val Gly Gly Asn Trp Val
1 5

<210> SEQ ID NO 20

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 61-69 of the TADG-14 protein

<400> SEQUENCE: 20

Val Leu Val Gly Gly Asn Trp Val Leu
1 5

<210> SEQ ID NO 21

<211> LENGTH: 9

<212> TYPE: PRT

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<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 49-57 of the TADG-14 protein

<400> SEQUENCE: 21

Ala Leu Phe Gln Gly Gln Gln Leu Leu
1 5

<210> SEQ ID NO 22
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 10-18 of the TADG-14 protein

<400> SEQUENCE: 22

Lys Thr Trp Met Phe Leu Leu Leu Leu
1 5

<210> SEQ ID NO 23
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 131-139 of the TADG-14 protein

<400> SEQUENCE: 23

Ser Leu Gly Ser Lys Val Lys Pro Ile
1 5

<210> SEQ ID NO 24
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 122-130 of the TADG-14 protein

<400> SEQUENCE: 24

Met Leu Leu Gln Leu Arg Asp Gln Ala
1 5

<210> SEQ ID NO 25
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 124-132 of the TADG-14 protein

<400> SEQUENCE: 25

Leu Gln Leu Arg Asp Gln Ala Ser Leu
1 5

<210> SEQ ID NO 26
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 170-178 of the TADG-14 protein

<400> SEQUENCE: 26

Thr Leu Asn Cys Ala Glu Val Lys Ile
1 5

<210> SEQ ID NO 27

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<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 208-216 of the TADG-14 protein

<400> SEQUENCE: 27

Cys Gln Gly Asp Ser Gly Gly Pro Leu
1 5

<210> SEQ ID NO 28
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 54-62 of the TADG-14 protein

<400> SEQUENCE: 28

Gln Gln Leu Leu Cys Gly Gly Val Leu
1 5

<210> SEQ ID NO 29
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 62-70 of the TADG-14 protein

<400> SEQUENCE: 29

Leu Val Gly Gly Asn Trp Val Leu Thr
1 5

<210> SEQ ID NO 30
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 191-199 of the TADG-14 protein

<400> SEQUENCE: 30

Gln Ile Thr Asp Gly Met Val Cys Ala
1 5

<210> SEQ ID NO 31
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 173-181 of the TADG-14 protein

<400> SEQUENCE: 31

Cys Ala Glu Val Lys Ile Phe Pro Gln
1 5

<210> SEQ ID NO 32
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 83-91 of the TADG-14 protein

<400> SEQUENCE: 32

Leu Gly Asp His Ser Leu Gln Asn Lys
1 5

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<210> SEQ ID NO 33
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 183-191 of the TADG-14 protein

<400> SEQUENCE: 33

Lys Cys Glu Asp Ala Tyr Pro Gly Gln
1 5

<210> SEQ ID NO 34
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 192-200 of the TADG-14 protein

<400> SEQUENCE: 34

Ile Thr Asp Gly Met Val Cys Ala Gly
1 5

<210> SEQ ID NO 35
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 71-79 of the TADG-14 protein

<400> SEQUENCE: 35

Ala Ala His Cys Lys Lys Pro Lys Tyr
1 5

<210> SEQ ID NO 36
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 113-121 of the TADG-14 protein

<400> SEQUENCE: 36

Asp Val Glu Asp His Asn His Asp Leu
1 5

<210> SEQ ID NO 37
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 229-237 of the TADG-14 protein

<400> SEQUENCE: 37

Gly Ser Asp Pro Cys Gly Arg Ser Asp
1 5

<210> SEQ ID NO 38
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 111-119 of the TADG-14 protein

<400> SEQUENCE: 38

Ser Ser Asp Val Glu Asp His Asn His

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1 5

<210> SEQ ID NO 39
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 28-36 of the TADG-14 protein

<400> SEQUENCE: 39

Ala Gln Glu Asp Lys Val Leu Gly Gly
1 5

<210> SEQ ID NO 40
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 217-225 of the TADG-14 protein

<400> SEQUENCE: 40

Val Cys Asp Gly Ala Leu Gln Gly Ile
1 5

<210> SEQ ID NO 41
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 241-249 of the TADG-14 protein

<400> SEQUENCE: 41

Val Tyr Thr Asn Ile Cys Arg Tyr Leu
1 5

<210> SEQ ID NO 42
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 247-255 of the TADG-14 protein

<400> SEQUENCE: 42

Arg Tyr Leu Asp Trp Ile Lys Lys Ile
1 5

<210> SEQ ID NO 43
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 7-15 of the TADG-14 protein

<400> SEQUENCE: 43

Arg Ala Ala Lys Thr Trp Met Phe Leu
1 5

<210> SEQ ID NO 44
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 42-50 of the TADG-14 protein

<400> SEQUENCE: 44

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His Ser Gln Pro Trp Gln Ala Ala Leu
1 5

<210> SEQ ID NO 45
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 48-56 of the TADG-14 protein

<400> SEQUENCE: 45

Ala Ala Leu Phe Gln Gly Gln Gln Leu
1 5

<210> SEQ ID NO 46
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 214-222 of the TADG-14 protein

<400> SEQUENCE: 46

Gly Pro Leu Val Cys Asp Gly Ala Leu
1 5

<210> SEQ ID NO 47
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 80-88 of the TADG-14 protein

<400> SEQUENCE: 47

Thr Val Arg Leu Gly Asp His Ser Leu
1 5

<210> SEQ ID NO 48
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 5-13 of the TADG-14 protein

<400> SEQUENCE: 48

Arg Pro Arg Ala Ala Lys Thr Trp Met
1 5

<210> SEQ ID NO 49
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 8-16 of the TADG-14 protein

<400> SEQUENCE: 49

Ala Ala Lys Thr Trp Met Phe Leu Leu
1 5

<210> SEQ ID NO 50
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 3-11 of the TADG-14 protein

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<400> SEQUENCE: 50

Arg Pro Arg Pro Arg Ala Ala Lys Thr
1 5

<210> SEQ ID NO 51

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 162-170 of the TADG-14 protein

<400> SEQUENCE: 51

Ser Pro Arg Glu Asn Phe Pro Asp Thr
1 5

<210> SEQ ID NO 52

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 188-196 of the TADG-14 protein

<400> SEQUENCE: 52

Tyr Pro Gly Gln Ile Thr Asp Gly Met
1 5

<210> SEQ ID NO 53

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 133-141 of the TADG-14 protein

<400> SEQUENCE: 53

Gly Ser Lys Val Lys Pro Ile Ser Leu
1 5

<210> SEQ ID NO 54

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 73-81 of the TADG-14 protein

<400> SEQUENCE: 54

His Cys Lys Lys Pro Lys Tyr Thr Val
1 5

<210> SEQ ID NO 55

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Residues 179-187 of the TADG-14 protein

<400> SEQUENCE: 55

Phe Pro Gln Lys Lys Cys Glu Asp Ala
1 5

<210> SEQ ID NO 56

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

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<220> FEATURE:
<223> OTHER INFORMATION: Residues 234-242 of the TADG-14 protein

<400> SEQUENCE: 56

Gly Arg Ser Asp Lys Pro Gly Val Tyr
1 5

<210> SEQ ID NO 57
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 246-254 of the TADG-14 protein

<400> SEQUENCE: 57

Cys Arg Tyr Leu Asp Trp Ile Lys Lys
1 5

<210> SEQ ID NO 58
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 101-109 of the TADG-14 protein

<400> SEQUENCE: 58

Val Gln Ser Ile Pro His Pro Cys Tyr
1 5

<210> SEQ ID NO 59
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 43-51 of the TADG-14 protein

<400> SEQUENCE: 59

Ser Gln Pro Trp Gln Ala Ala Leu Phe
1 5

<210> SEQ ID NO 60
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 6-14 of the TADG-14 protein

<400> SEQUENCE: 60

Pro Arg Ala Ala Lys Thr Trp Met Phe
1 5

<210> SEQ ID NO 61
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 26-34 of the TADG-14 protein

<400> SEQUENCE: 61

Ser Arg Ala Gln Glu Asp Lys Val Leu
1 5

<210> SEQ ID NO 62
<211> LENGTH: 9

-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 126-134 of the TADG-14 protein

<400> SEQUENCE: 62

Leu Arg Asp Gln Ala Ser Leu Gly Ser
1 5

<210> SEQ ID NO 63
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 149-157 of the TADG-14 protein

<400> SEQUENCE: 63

Gly Gln Lys Cys Thr Val Ser Gly Trp
1 5

<210> SEQ ID NO 64
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 96-104 of the TADG-14 protein

<400> SEQUENCE: 64

Gln Glu Ile Pro Val Val Gln Ser Ile
1 5

<210> SEQ ID NO 65
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 171-179 of the TADG-14 protein

<400> SEQUENCE: 65

Leu Asn Cys Ala Glu Val Lys Ile Phe
1 5

<210> SEQ ID NO 66
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 184-192 of the TADG-14 protein

<400> SEQUENCE: 66

Cys Glu Asp Ala Tyr Pro Gly Gln Ile
1 5

<210> SEQ ID NO 67
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 114-122 of the TADG-14 protein

<400> SEQUENCE: 67

Val Glu Asp His Asn His Asp Leu Met
1 5

-continued

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<210> SEQ ID NO 68
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 101-109 of the TADG-14 protein

<400> SEQUENCE: 68

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Val Gln Ser Ile Pro His Pro Cys Tyr
1             5

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<210> SEQ ID NO 69
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 236-244 of the TADG-14 protein

<400> SEQUENCE: 69

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Ser Asp Lys Pro Gly Val Tyr Thr Asn
1             5

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<210> SEQ ID NO 70
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 164-172 of the TADG-14 protein

<400> SEQUENCE: 70

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```

Arg Glu Asn Phe Pro Asp Thr Leu Asn
1             5

```

```

<210> SEQ ID NO 71
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Residues 174-182 of the TADG-14 protein

<400> SEQUENCE: 71

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```

Ala Glu Val Lys Ile Phe Pro Gln Lys
1             5

```

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<210> SEQ ID NO 72
<211> LENGTH: 1343
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense sequence of TADG-14

```

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<400> SEQUENCE: 72

gaattccgtg agtttattaa gggagatcta gtgcttatcc tagaatcagc          50
ccttgctgcc tatgatcttc ttgatccagt ccaggtagcg gcagatgttg          100
gtatagacgc caggtttgtc ggacctccca caggggtctg agccccagga          150
tgtgatgccc tggagtgcac catcacacac cagggggcct ccagaatcgc          200
cctggcacgt gtcagcccct ttgtgctgc ctgcacagac catgccatct          250
gtgatctgcc ccgggtaagc atcctcacac ttcttctggg gaaagatttt          300
tacttctgca cagttgagag tgtcaggaaa attctctcgg ggactggtga          350
cagtgcacca gcctgagacg gtgcacttct ggccaggctg ggtgcaatga          400

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-continued

tctgccaggc tgatgggctt cactttggac cccagggatg cctggtcacg	450
cagttgaaga agcatcagat catgggtgtg gtcctccaca tcgctgctgt	500
tgtagcaggg gtgtgggatg gactgaacca caggtatttc ttgctctggg	550
ccatctttat tctgtaggct gtggtctccc aggcgtaactg tgtatttcgg	600
ttttttacag tgggcagctg taaggaccca gttgccacct acaaggacac	650
cgccacagag tagttgctgg ccctggaaca aggccgcctg ccaaggctgc	700
gaatgggggtt ggcaactcatg acccccagc accttgctct cctgtgccct	750
ggagtgtcct gccagggctc ccccagcaa gagcaggaac atccacgtct	800
tggccgcacg aggtcggggg cgtcccatgg tgaggtcttc cagaatccag	850
ggcattccct gaaacctatc ctccctgaat ttgcctgttg gaggccctta	900
gttttccac tgaccaatgt gtaattggga ttcaaagcta tacctgaatt	950
tcctacatct ctagtaacag aatttaccaa acatgaactt tctgatggtg	1000
attacgtaac ttctaatttg aagccctttc ctcatctgtg ttttatgtcc	1050
aagaaatgtc tactgatttt gaaagatttg cttcacatc catcactcag	1100
atctaagttt tttctctcaa gtatggactg ttttatatcc tgtaagagct	1150
gaactacctt gacactggag cctttcttgc ttgctatgat tgaaaatcac	1200
atagaaagga ttctcataa tcctctagga tggtttcatt tacactgtgt	1250
aaattacaag ccatgacccc ctacattctt acattcataa ggtatttctt	1300
ccatttgagt tcggagagac ttggttaagct ctgcctgcta cag	1343

<210> SEQ ID NO 73
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sense primer for TADG-14, exon 2 to exon 3

<400> SEQUENCE: 73

cgacctcgtg cggccaagac g	21
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<210> SEQ ID NO 74
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Antisense primer for TADG-14, exon 2 to exon 3

<400> SEQUENCE: 74

cagctgtaag gacccagttg c	21
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<210> SEQ ID NO 75
 <211> LENGTH: 305
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: TADG-14 variant protein

<400> SEQUENCE: 75

Met Gly Arg Pro Arg Pro Arg Ala Ala Lys Thr Trp Met Phe Leu
1 5 10 15

-continued

Leu	Leu	Leu	Gly	Gly	Ala	Trp	Ala	Ala	Cys	Gly	Ser	Leu	Asp	Leu
			20					25					30	
Leu	Thr	Lys	Leu	Tyr	Ala	Glu	Asn	Leu	Pro	Cys	Val	His	Leu	Asn
		35					40					45		
Pro	Gln	Trp	Pro	Ser	Gln	Pro	Ser	His	Cys	Pro	Arg	Gly	Trp	Arg
	50					55					60			
Ser	Asn	Pro	Leu	Pro	Pro	Ala	Ala	Gly	His	Ser	Arg	Ala	Gln	Glu
65					70				75					
Asp	Lys	Val	Leu	Gly	Gly	His	Glu	Cys	Gln	Pro	His	Ser	Gln	Pro
			80					85						90
Trp	Gln	Ala	Ala	Leu	Phe	Gln	Gly	Gln	Gln	Leu	Leu	Cys	Gly	Gly
		95					100						105	
Val	Leu	Val	Gly	Gly	Asn	Trp	Val	Leu	Thr	Ala	Ala	His	Cys	Lys
		110					115					120		
Lys	Pro	Lys	Tyr	Thr	Val	Arg	Leu	Gly	Asp	His	Ser	Leu	Gln	Asn
	125					130					135			
Lys	Asp	Gly	Pro	Glu	Gln	Glu	Ile	Pro	Val	Val	Gln	Ser	Ile	Pro
140					145				150					
His	Pro	Cys	Tyr	Asn	Ser	Ser	Asp	Val	Glu	Asp	His	Asn	His	Asp
			155					160						165
Leu	Met	Leu	Leu	Gln	Leu	Arg	Asp	Gln	Ala	Ser	Leu	Gly	Ser	Lys
		170						175					180	
Val	Lys	Pro	Ile	Ser	Leu	Ala	Asp	His	Cys	Thr	Gln	Pro	Gly	Gln
		185					190					195		
Lys	Cys	Thr	Val	Ser	Gly	Trp	Gly	Thr	Val	Thr	Ser	Pro	Arg	Glu
	200					205					210			
Asn	Phe	Pro	Asp	Thr	Leu	Asn	Cys	Ala	Glu	Val	Lys	Ile	Phe	Pro
215					220					225				
Gln	Lys	Lys	Cys	Glu	Asp	Ala	Tyr	Pro	Gly	Gln	Ile	Thr	Asp	Gly
			230						235					240
Met	Val	Cys	Ala	Gly	Ser	Ser	Lys	Gly	Ala	Asp	Thr	Cys	Gln	Gly
		245						250					255	
Asp	Ser	Gly	Gly	Pro	Leu	Val	Cys	Asp	Gly	Ala	Leu	Gln	Gly	Ile
		260					265					270		
Thr	Ser	Trp	Gly	Ser	Asp	Pro	Cys	Gly	Arg	Ser	Asp	Lys	Pro	Gly
	275					280					285			
Val	Tyr	Thr	Asn	Ile	Cys	Arg	Tyr	Leu	Asp	Trp	Ile	Lys	Lys	Ile
290					295					300				
Ile	Gly	Ser	Lys	Gly										
			305											

What is claimed is:

1. A kit for detecting one or both of a TADG-14 protein or a TADG-14 mRNA, comprising one or both of:
 - an antibody specific for the TADG-14 protein or fragment thereof; or
 - an oligonucleotide probe with a DNA sequence complementary to a DNA encoding the TADG-14 protein.
2. The kit of claim 1, further comprising one or both of a means to detect the antibody or means to label the probe.
3. The kit of claim 1, wherein the antibody is specific for a TADG-14 protein sequence shown in SEQ ID NO: 7 or a TADG-14 protein variant sequence shown in SEQ ID NO: 75.

4. The kit of claim 1, wherein the oligonucleotide probe sequence is complementary to SEQ ID NO: 6.

5. The antibody comprising the kit of claim 1.

6. A method of detecting TADG-14 protein in a biological sample, comprising:

contacting the biological sample with the antibody of claim 5 or a fragment thereof; and

detecting binding of the antibody to TADG-14 protein or TADG-14 protein variant in the sample to indicate the presence of the TADG-14 protein or TADG-14 protein variant in the sample.

7. The method of claim 6, wherein the biological sample is blood, interstitial fluid, ascites fluid, tumor tissue biopsy, or circulating tumor cells.

8. The method of claim 6, wherein the biological sample is from an individual.

9. An isolated and purified TADG-14 protein variant, said TADG-14 variant has the amino acid sequence of SEQ ID NO. 75 or a fragment thereof.

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

obtaining a biological sample from an individual; and
detecting in the sample the TADG-14 protein variant of claim 9, wherein the presence of TADG-14 variant in the sample is indicative of the presence of carcinoma in the individual.

11. The method of claim 10, wherein the biological sample is blood, interstitial fluid, ascites fluid, tumor tissue biopsy, or circulating tumor cells.

12. The method of claim 10, wherein the cancer is an ovarian cancer, a breast cancer, a lung cancer, a colon cancer, or a prostate cancer in which TADG-14 protein variant is overexpressed.

13. The method of claim 10, wherein detection of the TADG-14 protein variant comprises a Northern blot, a Western blot, PCR, a dot blot, an ELISA sandwich assay, a radioimmunoassay, a DNA array chips, or flow cytometry.

14. A method of inhibiting TADG-14 in a cell at the mRNA or protein level, comprising:

introducing a TADG-14 antisense DNA or TADG-14 specific antibody or fragment thereof into the cell, wherein the TADG-14 antisense DNA hybridizes to endogenous TADG-14 mRNA or the TADG-14 antibody binds to TADG-14 protein, thereby inhibiting TADG-14 in the cell.

15. The method of claim 14, wherein the TADG-14 antisense DNA is introduced via a vector comprising: regulatory elements necessary for expressing DNA in the cell; and

DNA with a sequence shown in SEQ ID NO: 6 positioned in reverse orientation relative to said regulatory elements.

16. An immunogenic composition comprising an immunogenic fragment of a TADG-14 protein with a nine amino acid sequence and an appropriate adjuvant.

17. The immunogenic composition of claim 16, wherein the TADG-14 protein immunogenic fragment is a sequence of nine amino acids.

18. The immunogenic composition of claim 17, wherein the 9 amino acid fragment sequence is shown in SEQ ID NOS. 17, 18, 41, 42, 47, 48, 53, 56, or 64.

19. An oligonucleotide having a sequence of SEQ ID No. 72 or a pharmaceutical composition thereof.

* * * * *

专利名称(译)	细胞外丝氨酸蛋白酶		
公开(公告)号	US20100291124A1	公开(公告)日	2010-11-18
申请号	US12/802335	申请日	2010-06-04
[标]申请(专利权)人(译)	OBRIEN TIMOTHY J UNDERWOOD LOWELL J BEARD JOHN 繁昌野合		
申请(专利权)人(译)	奥布莱恩TIMOTHY J UNDERWOOD LOWELL J BEARD JOHN 繁昌野合		
当前申请(专利权)人(译)	生物仿制药, LLC		
[标]发明人	OBRIEN TIMOTHY J UNDERWOOD LOWELL J BEARD JOHN SHIGEMASA KAZUSHI		
发明人	O'BRIEN, TIMOTHY J. UNDERWOOD, LOWELL J. BEARD, JOHN SHIGEMASA, KAZUSHI		
IPC分类号	A61K39/00 G01N33/53 C07K14/47 C40B30/04 C12Q1/68 C12N5/071 C07H21/04 A61P35/00		
CPC分类号	C07K14/4748 C12N9/6424 G01N2500/00 G01N2333/96433 G01N33/57407		
其他公开文献	US8216794		
外部链接	Espacenet USPTO		

摘要(译)

本发明提供了编码新的细胞外丝氨酸蛋白酶的DNA，称为肿瘤抗原衍生的基因-14 (TADG-14)，其在卵巢癌，乳腺癌和结肠癌样品中过表达。还提供了能够表达本发明DNA的载体和宿主细胞，以及本发明的DNA和蛋白质的用途。还提供了TADG-14蛋白变体，其具有检测和靶向卵巢癌的潜在作用。

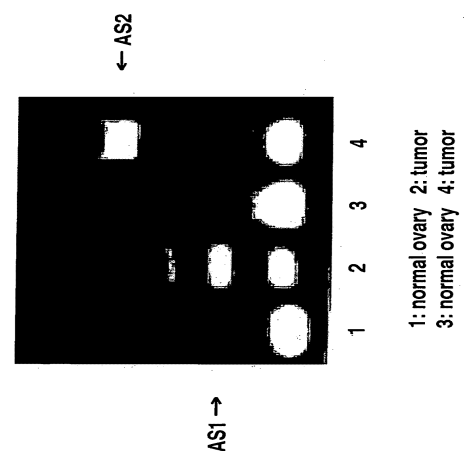


FIG. 1