

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2009207926 B2**

(54) Title
A new metastatic human tumor associated molecule, methods to detect both activated gene and protein and to interfere with gene expression

(51) International Patent Classification(s)
G01N 33/574 (2006.01)

(21) Application No: **2009207926** (22) Date of Filing: **2009.01.26**

(87) WIPO No: **WO09/092385**

(30) Priority Data

(31) Number	(32) Date	(33) Country
61/062,453	2008.01.25	US

(43) Publication Date: **2009.07.30**

(44) Accepted Journal Date: **2014.06.19**

(71) Applicant(s)
HANSABIOMED OU

(72) Inventor(s)
Lozupone, Francesco;Fais, Stefano

(74) Agent / Attorney
AJ PARK, L 11 60 Marcus Clarke St, Canberra, ACT, 2601

(56) Related Art
WO 2006/110593
EP 1104771

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 July 2009 (30.07.2009)

PCT

(10) International Publication Number
WO 2009/092385 A1

(51) International Patent Classification:
G01N 33/574 (2006.01)

(21) International Application Number:
PCT/EE2009/000001

(22) International Filing Date: 26 January 2009 (26.01.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/062,453 25 January 2008 (25.01.2008) US

(71) Applicant (for all designated States except US): **HANS-ABIONED OÜ** [EE/EE]; Akadeemia tee 19, EE12618 Tallinn (EE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **LOZUPONE, Francesco** [IT/IT]; Via Gran Sasso 48, I-00141 Roma (IT). **FAIS, Stefano** [IT/IT]; Via Svetonio 25, I-00136 Roma (IT).

(74) Agent: **KOPPEL, Mart Enn**; Patendibüroo Koppel OÜ, Kajakas 4-10, EE11317 Tallinn (EE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

(54) Title: A NEW METASTATIC HUMAN TUMOR ASSOCIATED MOLECULE, METHODS TO DETECT BOTH ACTIVATED GENE AND PROTEIN AND TO INTERFERE WITH GENE EXPRESSION

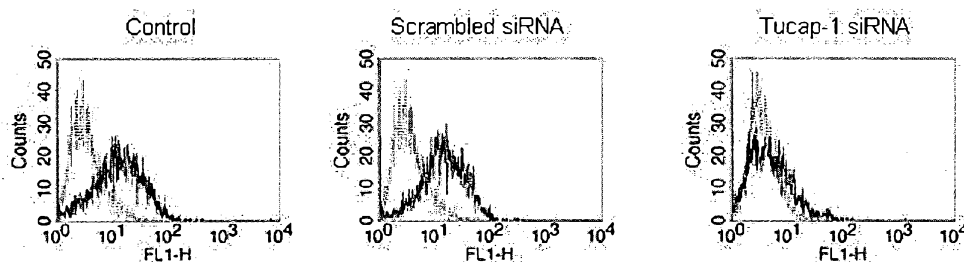


Figure 8

(57) Abstract: This disclosure characterizes the function and the expression of the human protein encoded by *tm9sf4*. The protein is highly expressed in malignant tumor cells and therefore is a novel marker for malignancy. Moreover, the protein is involved in the phagocytotic character of tumor cells. This disclosure provides methods and tools to diagnose and follow up malignancy of tumors. Furthermore, means to inhibit phagocytotic character of tumor cells as well as means to treat cancer are provided.

NEW METASTATIC HUMAN TUMOR ASSOCIATED MOLECULE, METHODS TO DETECT BOTH ACTIVATED GENE AND PROTEIN AND TO INTERFERE WITH GENE EXPRESSION

TECHNICAL FIELD

- 5 The present invention relates generally to the field of cancer research and more specifically to identification of new tumorigenesis and metastasis related proteins and genes. The invention relates to a new marker of malignancy. The invention further relates to the field of gene therapy.

BACKGROUND ART

- 10 In 2005, 7.6 million people died of cancer out of 58 million deaths worldwide. Based on projections, cancer deaths will continue to rise with an estimated 9 million people dying from cancer in 2015, and 11.4 million dying in 2030 (WHO data). Cancer treatment may involve surgery, radiation therapy, chemotherapy, hormonal therapy, or some combination of these, but presently survival rates for the most of cancer patients is very low. According to World Health Organization, one third of the cancer burden could be cured if detected early and treated
- 15 adequately. Pathological research provides means for establishing the diagnosis of the most of solid tumors. Although many cases can be classified reliably with current pathological criteria, there is a significant subset of cases in which no consensus can be reached even among expert pathologists. Diagnostic ambiguity has significant adverse consequences for patients. Misclassifying a tumor as benign may be fatal, and diagnosing a benign lesion as malignant may
- 20 result in significant morbidity. Currently there is no method to definitively resolve these ambiguities. Therefore, there is a clear need for a diagnostic test that could reduce these uncertainties.

- Phagocytosis is the process by which cells internalize large particles (typically 0.1 mm diameter), such as bacteria or cell debris. The early stage of phagocytosis can be tentatively divided into
- 25 distinctive steps: cell membrane binding around the particle, phagosome formation, and internalization of the phagosome. In the process of phagosome formation and internalization, actin cytoskeleton has been proposed to drive these steps to allow engulfment.

Phagocytic cells have been identified in malignant tumors up to a century ago, and, more recently, cells with phagocytic behavior (also defined as cannibalistic behavior) have been

29 May 2014
2009207926

detected in tumors of different histology, such as oat cell carcinoma of the lung, breast cancer, bladder cancer, medulloblastoma, gastric adenocarcinomas, melanoma and squamous cell carcinoma of the skin.

We have recently observed that phagocytosis is a character of metastatic melanoma cells able to phagocytose apoptotic cells, plastic beads stained yeasts, and live lymphocytes displaying efficient phagocytic machinery responsible for a macrophage-like activity, while melanoma cells derived from primary lesions did not display any cannibalistic or phagocytic activity. Moreover, cannibal cells can be detected in 100% metastatic melanoma lesions (Lugini et al., 2004; Lugini et al., 2006).

One of the main features of cannibal cells is an increased acidity of lysosomal-like vesicles and an over expression of cathepsin B, a proteolytic enzyme reported to be involved in tumor invasion and metastasis (Sloane et al., 1981) . Differently from professional phagocyte -like macrophages, cannibal tumor cells do not utilize structures like ruffles or any pseudopodial movement. Instead, live or dead material that touches the tumor cell's external membrane is immediately endocytosed and digested through a sort of quicksand mechanism that seems not to involve any specific receptor.

These findings have led us to speculate that cannibal cells feed of other cells, perhaps with no particular need of blood-derived nutrient supply, but also that cannibalism of lymphocytes by tumor cells may represent a rudimentary mechanism of tumor immune escape. Moreover, these findings led us to a novel, revolutionary interpretation that cancer cells, in their habit to use other cells for feeding, may behave as unicellular eukaryotes whose unique purpose is to survive in a continuous fighting against other cells and the unfavourable environment. This theory further led us to speculate that amoebas and metastatic cells might share the same framework with the same regulatory elements allowing their surviving in adverse micro-environmental conditions. However, so far no genes have ever been specifically associated with the cannibal behaviour of cancer cells.

The cellular slime mold *Dictyostelium discoideum* has been previously used as a model organism to study phagocytosis. Mechanisms involved in phagocytosis by Dictyostelium cells are very similar to those used by mammalian phagocytes, and involve the actin cytoskeleton and RacFl, a member of the Rho family of GTPbinding proteins. However, no phagocytosis associated specific proteins have ever been identified in mammals.

It has been recently found that the protein encoded by *PHGLA* gene was implicated in cell adhesion and phagocytosis in the amoeba *Dictyostelium discoideum*. This protein belongs to TM9 superfamily and genes encoding TM9 proteins can be unambiguously identified in eukaryotic genomes. The family includes many members in organisms ranging from yeast
5 to plants and human. To mention some example, there are three members of this family in *Saccharomyces cerevisiae*, *Dictyostelium amoebae*, and *Drosophila* flies and four in humans and mice. All of them exhibit a similar overall structure, with a rather variable potential luminal domain followed by a more conserved membrane domain and nine or ten putative transmembrane domains.

- 10 In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of
15 the common general knowledge in the art.

In the description in this specification reference may be made to subject matter that is not within the scope of the claims of the current application. That subject matter should be readily identifiable by a person skilled in the art and may assist in putting into practice the invention as defined in the claims of this application.

20 SUMMARY OF THE INVENTION

- Based on our theory that cancer cells use other cells for feeding and behaving as unicellular eukaryotes and possibly share the same framework with the same regulatory elements as amoebas, we compared *PHGLA* gene with human genome. Three homologues of *phgl* have been fully sequenced in human (TM9SF4, U81006 and U94831), and we found the closest
25 homologue of *phgl* of *Dictyostelium dicoideum* in human to be tm9sf4 (other aliases: KIAA0255, dJ836N17.2) locating in chromosome 20ql 1.21. Even if this gene is fully sequenced, its function or expression product of this has never been characterized.

- Accordingly, we have studied the function and the expression of the protein encoded by *tm9sf4* in more details. In this disclosure, we show the function of the protein and provide a
30 number of applications.

This disclosure shows that the protein is highly expressed in malignant cells. Observations on several melanoma cell lines deriving from patients show that TM9SF4 is a marker of malignancy, since this protein is undetectable on the cell lines deriving from primary lesions. Furthermore, this protein is involved in the phagocytic behavior of metastatic melanoma cells, since silencing the gene encoding this protein strongly inhibits the phagocytic behavior of metastatic cells. Based on these observations we have named this protein as TUCAP-I (Tumor associated cannibal protein Y). The gene coding for this protein is here accordingly called *tucap-1* gene.

This invention addresses the need for a rapid test to detect malignant cells and diagnose melanoma and other tumors; and/or at least provides the public with a useful choice. Accordingly, this disclosure provides antibodies and markers useful for TUCAP-I detection, analysis and potential therapeutic effects.

Also described are polynucleotides corresponding or complementary to all or part of the *tucap-1* gene, and polynucleotides or oligonucleotides, which hybridize to the *tucap-1* gene, mRNAs, or to TUCAP-1 -encoding polynucleotides. Recombinant DNA molecules containing TUCAP-1 polynucleotides, cells transformed or transduced with such molecules, and host-vector systems for the expression of *tucap-1* gene products are described. The disclosure further describes TUCAP-1 protein and polypeptide fragments thereof.

Also described are antibodies that bind to TUCAP-1 proteins and polypeptide fragments thereof, including polyclonal and monoclonal antibodies, murine and other mammalian antibodies, chimeric antibodies, humanized and fully human antibodies, and antibodies labelled with a detectable marker, and antibodies conjugated to radionucleotides, toxins or other therapeutic compositions. Further described are methods for detecting the presence of TUCAP-1 polynucleotides and proteins in various biological samples, as well as methods for identifying cells that express TUCAP- 1.

Accordingly, in one aspect the invention provides a marker for tumor malignancy, said marker consisting of amino acid sequence according to SEQ ID NO:11; SEQ ID NO:12 or SEQ ID NO:15..

In another aspect, the invention provides an isolated antibody, generated by immunizing mice, rabbit, goat or donkey with a peptide fragment having an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12 and SEQ NO: 15, wherein the antibody is capable of recognizing human TUCAP-1 protein by binding to a peptide fragment selected from the group consisting of amino acids 18-282, 221-235 and 303-352 of SEQ ID NO:1.

In another aspect, the invention provides monoclonal antibodies against human TUCAP-1 protein, said antibodies having a tumor growth blocking activity or metastasis blocking activity, said antibodies being generated by immunizing mice with a peptide fragment selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:15, and said antibodies recognizing human TUCAP-1 protein by binding to conformational or linear epitopes locating between aa 18 to 282, or 221-235 or 303-352 of SEQ ID NO:1.

In another aspect, the invention provides hybridomas obtained by using spleen cells of mice immunized with a peptide fragment having an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12 and SEQ NO: 15, and producing monoclonal antibodies, or fragments or humanized forms of such monoclonal antibodies or single chain forms of the fragments capable of binding to conformational or linear epitopes of the human TUCAP-1, said epitopes locating between amino acids 18- 282, or 221-235 or 303-352 of SEQ ID NO:1.

The invention also provides a kit for detecting malignant tumors *in vitro*, said kit comprising antibodies capable of recognizing human TUCAP-1 protein by binding to a peptide fragment selected from the group consisting of amino acids 18-282, 221-235 and 303-352 of SEQ ID NO:1 ; and a titred positive control comprising a peptide fragment having an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 15, wherein the antibody is used to detect an oncogenic protein according to SEQ ID NO: 1 from tissues and body fluids of patients with tumors. The invention also relates to a method to distinguish metastatic tumors *in vitro* from non-metastatic tumors, said method comprising a step of defining presence or absence of expression of SEQ ID NO: 1 in tumor cell, wherein presence of the expression indicates metastatic character of the cell.

The invention also relates to a method to distinguish metastatic tumors *in vitro* from non-metastatic tumors, said method comprising a step of defining presence or absence of expression of SEQ ID NO: 1 in tumor cell, wherein presence of the expression indicates metastatic character of the cell.

- 5 The invention also relates to a method to follow up metastatic activity of a tumor, said method comprising a step of defining presence or absence of expression of SEQ ID NO: 1 in tumor cell, wherein presence of expression indicates metastatic activity.

The invention also relates to a method of inhibiting phagocytotic character of tumor cells, said method comprising a step to inhibit expression of SEQ ID NO: 1.

- 10 The invention also relates to a method to treat metastatic tumors, said method comprising a step of administering to a patient a therapeutically effective amount of a composition comprising a TUCAP-1 binding agent conjugated to a chemotherapeutic drug.

The invention also relates to the use of a TUCAP-1 binding agent conjugated to a chemotherapeutic drug for the manufacture of a medicament for treating metastatic tumors.

15 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Molecular structure of TM9SF4/TUCAP-1 protein.

(A) Hydropathy profile of TUCAP-1 protein sequence. Hydrophobic regions are indicated above the line by positive values. Amino acid numbering is indicated on the abscissa. Hydrophilic stretch in the N-terminal region is followed by nine hydrophobic regions.

- 20 Analysis was performed according to Claros and von Heijne using TopPred prediction Program.

(B) Graphic representation of TUCAP-1 secondary structure according to TopPred predictor server.

- 25 Figure 2. Detection of TM9SF4/TUCAP-1 transcripts and characterization of TUCAP-1 antibodies.

(A) RT-PCR analysis of TUCAP-1 (upper panel) and GAPDH (lower panel) on five metastatic (MM1-5) and five primary melanoma (PM1-5) cell lines recently established in

vitro from metastatic lesion, and peripheral blood cells from two different donors (PBL1 - 2); M size marker.

(B) Western blots of 6-histidine or TUCAP-I in the six-histidine tagged TUCAP-I peptide used to immunize mice and in uninduced bacterial lysates. Equal amount of purified
5 protein and bacterial lysate was loaded on reducing gels and blotted with the 6-His-antibody or TUCAP-I mice antisera. Proteins were visualized using HRP-conjugated secondary antibodies and revealed with ECL system (Pierce).

(C) Western blotting for GFP-TUCAP-I and GAPDH on Triton soluble (lane 1) and Triton insoluble (lane 2) fractions of GFP-TUCAP-I (GFP-Tuc) transfected MM1 cells, and Triton
10 soluble and insoluble fractions of untransfected MM1 cells (lanes 3-4). M size marker. Proteins were visualized using HRP conjugated secondary antibodies and revealed with ECL (Pierce). As molecular weight markers Rainbow™ (Amersham UK) prestained standards were used.

Figure 3. Western blotting analysis of TUCAP-I.

15 Western blotting for TUCAP-I detection on GFP-Tuc Transfected MM2 cells, Four metastatic melanoma cell lines (MM2-5), and CCD-1064SK human skin fibroblasts (HSC). Loading amount was controlled by immunodetection of actin. Proteins were visualized using HRP conjugated secondary antibodies and DAB system (DAKO, Denmark) as cromogen. Rainbow™ (AmershamUK) prestained standards were used as molecular
20 weight markers.

Figure 4. Immuno-cytochemical and immuno-histochemical analysis of TUCAP-I. Figure 4A is the same in black and white.

Mice pre-immune serum immunocytochemical analysis of (A) MM2 cells; (B) peripheral blood lymphocytes; (C) *in vitro* differentiated macrophages.

25 TUCAP-I immunocytochemical analysis of: (D) M2 cells; (E) peripheral blood cells; (F) Macrophages.

Immunohistochemical analysis of malignant melanoma tissues stained with: (G) preimmune mouse serum; (H) TUCAP-I immune serum; and (I) anti-GP100.

Immunohistochemical analysis of healthy skin stained with: (J) mouse preimmune serum,
30 (K)TUCAP-I immune serum, and (L) anti-ezrin antibody. Magnification 10X.

Figure 5. Subcellular localization of TUCAP-I/ - 1. Figure 5 A is the same in black and white.

(A) Western blot analysis of subcellular fractions of TUCAP-I immunoprecipitates from MM2 total lysates. M size marker, TE total extracts.

- 5 (B) Immunofluorescence (IF) double staining analysis of TUCAP-I (green) and Rab5 (Red). (C) IF double staining analysis of TUCAP-I (green) and Lamp-1 (red). (D) IF double staining analysis of TUCAP-I (green) and Mitotracker stained mitochondria (red). Yellow/orange areas indicate co-localization. Nuclei were stained with Hoechst 33258. Magnification 100X.

- 10 Figure 6. Subcellular localization of TUCAP-I - II. Figure 6 A is the same in black and white. Immunofluorescence (IF) double staining analysis of TUCAP-I (green) and EEAI (Red) on MM2 cells. Yellow/orange areas indicate co-localization. Nuclei were stained with Hoechst 33258. Magnification 100X.

Figure 7. TUCAP-I detection in cannibal cells. Figure 7A is the same in black and white.

- 15 (A) Detection and localization of TUCAP-I in metastatic melanoma MM1 cells co-cultured with living lymphocytes. IVM analysis of TUCAP-I (green). Picture highlights that TUCAP-1 is detectable exclusively on melanoma cells.
- (B) Double fluorescence analysis of TUCAP-I (green) and EEA-I (red) in metastatic melanoma MM1 cells co-cultured with living lymphocytes. Yellow/orange areas indicate
- 20 co-localization. Nuclei are stained with Hoechst 33258.

Figure 8. FACS analysis of TUCAP -1 expression.

Facs analysis of TUCAP-I expression in untransfected MM2 cells, Scrambled siRNA and TUCAP-I siRNA (TM9SF4 siRNA) transfected MM2 cells 48 hours after transfection.

Figure 9. Functional analysis of TM9SF4/TUCAP-1.

- 25 (A) FACS analysis of phagocytic activity of Scrambled siRNA (SC-siRNA) or TUCAP-I siRNA (TM9SF4 siRNA) transfected MM2 cells. Gray: unstained control cells; red: negative control Scrambled siRNA transfected cells; green: TM9SF4 siRNA transfected cells.
- (B) FACS analysis of cannibal activity of SC-siRNA or TM9SF4 siRNA transfected MM2
- 30 cells incubated 18 hours with DHR123-stained lymphocytes. Gray: unstained control cells;

29 May 2014

2009207926

red: SC-siRNA transfected cells; Green: TM9SF4 siRNA transfected. Cells. To exclude DHR123-stained lymphocytes not ingested with melanoma cells, exclusively melanoma cell fluorescence emission was evaluated.

(C) FACS analysis of LysoTracker DND-26 staining of SC-siRNA or TM9SF4 siRNA transfected MM2 cells. Gray: unstained control cells; red: SC-siRNA transfected; Green: TM9SF4 siRNA transfected.

Figure. 10. TUCAP-I overexpression enhance cell invasion through Matrigel. Figure 10A is the same in black and white. Phase micrograph of invading WM743 melanoma cells as compared to GFP-Tagged full length TUCAP-I WM743 melanoma cells (TWM). Invading cells were Fixed in formaldehyde and stained with crystal violet. Picture clearly show that the number of invading cells was significantly higher for TWM , with a mean of 25 cells for untransfected versus a mean of 132 cells for TUCAP 1 transfected TWM.

DESCRIPTION OF THE INVENTION

TUCAP-1/TM9SF4 (HUGO nomenclature official committee official full name: transmembrane 9 superfamily protein member 4) belongs to the transmembrane 9 superfamily (TM9SF), a highly conserved family of proteins characterized by the presence of a large variable extracellular N-terminal domain and nine to ten putative transmembrane domains. However, function and localization of TM9SF4 has never been described in human cells. In this disclosure, we localize the protein expression and provide useful and novel applications to this protein. This disclosure identifies the protein as a novel oncoprotein and shows that the protein is expressed in malignant tumor cells while undetectable in variety of healthy cells and tissues. This disclosure also shows the structure of the protein and by analogy with other proteins shows that the protein may be involved in pH regulation of intracellular vesicles. Throughout this disclosure we call the protein TUCAP-I and the gene coding for the protein is accordingly called *tucap-1*.

Table 1 below shows the physicochemical parameters of TUCAP-1 protein

Tupac-1 ProtParam computation of physicochemical parameters:	
Number of amino acids:	625
Molecular weight:	72541.3
Theoretical pI:	6.22
Extinction coefficients:	132880 Abs 0.1% (=1 g/l) 1.832, assuming ALL Cys residues appear as half cystines
	132130 Abs 0.1% (=1 g/l) 1.821, assuming NO Cys residues appear as half cystines
Estimated half-life:	30 hours (mammalian)

Hydropathy analysis of TUCAP-I through TopPred predictor server revealed a mostly hydrophilic, amino terminal portion that extends up to amino-acid 262, while the remaining portion of the protein is extremely hydrophobic and contains nine potential transmembrane domains (Fig. 1A) On the basis of this predicted structure it may be hypothesized with great confidence that TUCAP-I is an integral membrane protein as shown in Fig. 1B Our finding that there is a fairly high homology between TUCAP-I and PHG1 (45% that rises to 63% if NCBI Blast protein program positive alignment is considered) led us to a novel hypothesis that TUCAP-I may have a role in cannibal activity of human metastatic melanoma cells.

According to one embodiment described are polynucleotides corresponding or complementary to all or part of the *tucap-1* gene, mRNAs, and/or coding sequences, preferably in isolated form, including polynucleotides encoding TUCAP-1 proteins and fragments thereof, DNA, RNA, DNA/RNA hybrid, and related molecules, oligonucleotides complementary to the *tucap-1* gene or mRNA sequences or parts thereof, and polynucleotides or oligonucleotides which hybridize to the TUCAP-1 genes, mRNAs, or to TUCAP-1 -encoding polynucleotides.

According to another embodiment described is an "antibody" which is a whole antibody molecule or fragment thereof that recognizes (or can bind to) "*specific sequences*" of TUCAP-1 protein, which is its antigen. Antibody may be either a polyclonal antibody or a monoclonal antibody. In this embodiment, TUCAP-1 protein is a polypeptide having the amino acid sequence according to SEQ ID NO:1, and the *specific sequences* are

polypeptides having an amino acid sequence containing deletion, substitution or addition of one or more amino acids as compared to the amino acid sequence of SEQ ID NO:1 or a fragment of these.

5 In one aspect, the invention provides an isolated antibody, generated by immunizing mice, rabbit, goat or donkey with a peptide fragment having an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12 and SEQ NO: 15, wherein the antibody is capable of recognizing human TUCAP-1 protein by binding to a peptide fragment selected from the group consisting of amino acids 18-282, 221-235 and 303-352 of SEQ ID NO:1.

10 The invention also provides the antibody of the invention, or fragment thereof, wherein the antibody is a monoclonal antibody and belongs to IgG isotype IgG2A, or IgG isotype IgG1.

The antibody of the present invention encompasses antibody mutants. An "antibody mutant" is a mutant in which one or more amino acid residues in the antibody have been
15 modified from the original.

According to further embodiments described are TUCAP-1 inhibitors. Such inhibitor molecules may be polynucleotide sequences that are substantially complimentary to the sequence of SEQ ID NO: 2 or part of it, and oligonucleotide sequences substantially complimentary to a fragment of SEQ ID NO:2.

20 According to yet another embodiment generally described are methods for treating cancer in a human patient comprising the step of administering to the patient a therapeutically effective amount of a composition comprising a TUCAP-I binding agent conjugated to a chemotherapeutic drug. Antibodies and fragments that specifically bind to TUCAP-I protein can be used to treat cancers. Also described is the use of antibodies and antibody
25 fragments that are fused to other moieties that can have a cytotoxic effect on cancer.

The invention provides a method to treat metastatic tumors, said method comprising a step of administering to a patient a therapeutically effective amount of a composition comprising a TUCAP-1 binding agent conjugated to a chemotherapeutic drug.

The invention also relates to a of a TUCAP-1 binding agent conjugated to a chemotherapeutic drug for the manufacture of a medicament for treating metastatic tumors.

Another embodiment described is cell lines producing monoclonal antibodies against TUCAP-I protein or fragments thereof.

- 5 The invention also provides hybridomas obtained by using spleen cells of mice immunized with a peptide fragment having an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12 and SEQ NO: 15, and producing monoclonal antibodies, or fragments or humanized forms of such monoclonal antibodies or single chain forms of the fragments capable of binding to conformational or linear epitopes of the
- 10 human TUCAP-1, said epitopes locating between amino acids 18- 282, or 221-235 or 303-352 of SEQ ID NO:1.

- According to yet another embodiment described are recombinant DNA or RNA molecules containing full-length wild type or mutated TUCAP-I sequence, or deletion mutants of TUCAP-I, including but not limited to phages, plasmids, phagemids, cosmids, YACs,
- 15 BACs, as well as various viral and non- viral expression vectors well known in the art, and cells transformed or transfected with such recombinant DNA or RNA molecules. Using these expression vectors, TUCAP-I may be preferably expressed in several malignant tumor cell lines. Preferred embodiments comprise host-vector systems that are useful for the production of a TUCAP-I protein or fragment thereof. Such host- vector systems may
- 20 be employed: i) to study the functional properties of TUCAP-I and TUCAP-I mutations; ii) as model to develop a gene therapy protocol based on the utilization of TUCAP-I silencing vectors and TUCAP-I mutants expressing vectors.

- Accordingly, this invention provides a unique tool to measure, study and detect TUCAP-I, as marker of malignancy of many cancer types. Moreover, this invention introduces a new
- 25 tool for clinical oncologist in the management and follow up of cancer patients. Moreover, peptides of other molecules able to interfere with the expression or acting as TUCAP-I blocking agents are described as antineoplastic compounds.

Yet another preferred embodiment of this invention is a kit to detect TUCAP-I from tissue specimens and body fluids of tumor patients as a diagnostic and or prognostic tool such as

a detection kit. Such kits generally comprise: a) anti TUCAP-I antibodies; b) a positive control consisting of the purified TUCAP-I protein, and the necessary buffers.

More specifically, the invention provides a kit for detecting malignant tumors *in vitro*, said kit comprising antibodies capable of recognizing human TUCAP-1 protein by binding to a
5 peptide fragment selected from the group consisting of amino acids 18-282, 221-235 and 303-352 of SEQ ID NO:1 ; and a titred positive control comprising a peptide fragment having an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 15, wherein the antibody is used to detect an
10 oncogenic protein according to SEQ ID NO: 1 from tissues and body fluids of patients with tumors.

The invention is now described by means of examples, which are not meant to limit the scope of the invention. The scope of the invention is defined by the appended claims.

EXAMPLE

EXAMPLE 1

15 **Tucap-1 transcripts and TUCAP-I protein are detectable in human malignant melanoma cells, but not in primary melanoma cells, in peripheral blood mononuclear cells or in healthy skin cells.**

Cell culture. Human primary and metastatic melanoma cell lines were respectively derived from primary or metastatic tumor lesions of patients surgically resected at the Istituto
20 Nazionale dei Tumori, Milan, Italy. All cells employed in the current study were designated by PM (primary melanoma) or MM (metastatic melanoma), followed by a progressive number. Human peripheral blood mononuclear cells (PBMC) were purified by Ficoll-Hypaque (Pharmacia) density gradient of buffy coats from healthy donors. Monocytes were separated from PBMC by using CD 14 labeled Miltenyi microbeads
25 according to manufacturer's indications and were left to differentiate for 2 weeks at 3 TC in RPMI 1640 plus 15% FCS. Remaining peripheral blood lymphocytes (PBL), were obtained after CD 14 beads mediated monocyte ablation. All the cells were seeded in RPMI 1640 supplemented with 100 IU/mL penicillin, 100 Ag/mL streptomycin, 10% FCS in a 5% CO2 environment at 3 TC. (All reagents were purchased from Cambrex).

29 May 2014
2009207926

PCR analysis. Expression of *Tucap-1* transcripts was assessed by rt-PCR on several primary and metastatic melanoma cell lines obtained from melanomas of patients surgically resected at Istituto Nazionale Tumori, Milan, as compared to peripheral blood lymphocytes (PBL). Total RNA from the cells was obtained by the RNazol (Invitrogen) method and RNA templates were used for RT-PCR amplification. Primers for TUCAP-1 detection were:

tgtgtgaaacaagcgccttc (SEQ ID NO: 3, and

atgaggtggacgtagtagt (SEQ ID NO: 4).

These primers amplify a fragment of 349 base pairs.

10 Primers used to direct TUCAP-1 His-tagged N-terminal domain synthesis were:

gaattcatgtgtgaaacaagcgcctt (SEQ ID NO:5) and

gtcgacagaaaaccagtggatctg (SEQ ID NO:6).

Primers to detect GAPDH were: ccatggagaaggctgggg (SEQ ID NO:7) and

caaagttgtcatggatgacc (SEQ ID NO: 8).

15 **TUCAP 1 cloning and expression of TUCAP 1 fusion protein in human melanoma cells:**

PCR products were cloned into pTopo vector (Invitrogen) and then excised with the appropriate pair of restriction enzymes (EcoRI, Sail) to acquire a single fragment that was subsequently ligated in the pTrcHis2 vector (Invitrogen). The expressed recombinant protein was purified employing Ni NTA agarose resin (Qiagen) following manufacturer's instructions and utilized to immunize mice.

Primers that were used to direct GFP-tagged full length TUCAP-I were:

gaattcatgtgtgaaacaagcg (SEQ ID NO:9), and

gtcgatgtctatcttcacagcata (SEQ ID NO: 10)

25 PCR products were cloned into pTopo vector (Invitrogen) and then excised with the appropriate couple of restriction enzymes (EcoRI-Sall) and ligated to acquire a single fragment that subsequently was ligated in the pEGFPN1 vector (Clontech) at the EcoRI and Sail sites to produce the GFP-TUCAP-I fusion protein. Plasmids encoding the GFP-TUCAP-1 fusion protein were transfected into MMI and MM2 cells by using the Lipofectamine

2000 transfection kit (Invitrogen) according to the manufacturer's instructions, thus obtaining GFP-TUCAP-I.

(GFP-Tuc) MM1 or MM2 cells. The percentage of transfected cells was evaluated by Fluorescence-activated cell sorting analysis.

5 **Western Blotting and immunoprecipitation**

Bacterial lysates, whole melanoma cell lysates and CCD-1064SK healthy skin fibroblasts (SantaCruz) were resuspended in SDS sample buffer, denaturated by boiling, separated by SDS-PAGE, and analyzed by Western blot. 6xHis tagged protein, GFP, TUCAP-I, and GAPDH, were respectively detected with anti6His mAb (Sigma), anti GFP (clone 1E4 MBL), anti TUCAP-I mouse serum and antiGAPDH (SantaCruz). TUCAP-I proteins were immunoprecipitated overnight at 4°C in the presence of protein A+G-Sepharose beads (Pierce) from precleared cell lysates, by using rabbit anti TUCAP-I pAb antibody. Rabbit preimmune serum was used as negative control. Actin was detected with anti actin mAb (Sigma).

- 15 In order to characterize *tucap-1* gene product, cDNA derived from MM1 cells was cloned in bacterial expression vectors to obtain TUCAP-1 first 265 amino acids fused to a 6-Histidine N-terminal tag (6H-Nt- TUCAP 1) (SEQ ID NO: 11) Western blot analysis of purified recombinant protein resulted in a translation product of about 30 kDa absent in control bacterial whole lysates (negative control). Therefore, His-tagged TUCAP-I
- 20 recombinant peptide was employed as immunogen to produce anti- TUCAP-I antibodies in mice. The specificity of the TUCAP-I antiserum was determined by Western blot analysis of the purified 6H-Nt- TUCAP-I immunoblotted with anti 6His and TUCAP-I mouse antisera (Fig. 2 B). TUCAP-I mouse antiserum was further analyzed by Western blot on Triton soluble and Triton insoluble fractions of MM1 cells transfected or not transfected
- 25 with a GFP-tagged full length TUCAP-I (GFP- TUCAP-I). The anti-GFP antibody revealed a single specific translation product in the 100 kDa range, while anti-TUCAP-1 antibodies recognized both the GFP-tagged and the endogenous TUCAP-I corresponding to a 70 kDa protein detectable in both cell lines (Fig. 2C). Interestingly, TUCAP-I was more represented in the Triton insoluble fractions (GAPDH negative, cytoskeletal proteins
- 30 enriched fraction), thus supporting the provisional models proposing TUCAP-I as a transmembrane protein. To further support PCR results, the anti-TUCAP-1 antibodies were blotted in cellular extracts of four metastatic melanoma cells (MM2-MM5), previously

analyzed for their cannibal behavior as compared to healthy skin fibroblasts (HSC) and GFP- TUCAP-I transfected MM2 cells, as a control. TUCAP-I was exclusively detectable in melanoma cells, while undetectable in skin cells (Fig. 3).

EXAMPLE 2

5 Immunochemistry shows TUCAP-I exclusively in melanoma cells

Immunocytochemistry and immunohistochemistry. For immunocytochemistry melanoma cells and macrophages, cultured on glass chamber slides (Falcon), and PBL, cytopun on glass slides, were fixed with 80% methanol 10 minutes at 4X2 and stained for TUCAP-I, TUCAP-I mouse serum or preimmune control serum. Malignant melanoma and
10 corresponding normal skin tissue from Biomax array slides (Biomax) were immunostained with pre-immune serum, for anti-TUCAP-1 mouse antiserum. Melanoma was also stained for anti-gp100 (Immunotech) while normal skin was also stained for anti-ezrin (Sigma). Proteins were visualized using the peroxidase antiperoxidase method in single staining (Dako) and counterstained with Mayer's hematoxylin.

15 Fig.4 A-C shows that MM2 cell lines (A), Peripheral blood lymphocytes (B), and in vitro differentiated Macrophages (C), were negative for mouse preimmune serum. Consistently with PCR results malignant melanoma cultured cells showed clear positive staining for TUCAP-I (Fig 4 D) while PBL (Fig. 4E) and macrophages (Fig. 4 F) were negative for TUCAP-I staining. Immunohistochemical analysis of malignant melanoma tissues as
20 compared to healthy skin suggested that TUCAP-I was detectable only in melanoma tissues (Fig. 4 H) while undetectable in healthy skin (4K). As positive control markers for melanoma and normal skin GP100 (Fig 4I), and ezrin (Fig. 4L) were used respectively. Pre-immune mouse serum staining was always negative in both tissues (Fig. 4G, 4J). These results provide clear evidence that TUCAP-I was exclusively detectable in melanoma cells
25 and thus support the results of Example 1.

EXAMPLE 3

Subcellular localization of TUCAP-I

Further experiments were performed to analyze the intracellular localization of TUCAP 1.

Cell compartment fractionation. Cells were harvested and processed according to Qproteome plasma membrane kit protocol (Quiagen) in order to obtain non denatured fractions of cellular compartments corresponding to purified plasma membranes and cytosol. The latter fractions were then precipitated with acetone and resuspended in immunoprecipitation buffer B (0.1% SDS, 1% NP40, 0.5% sodium cholate) in order to be subjected to immunoprecipitation with rabbit anti TUCAP-I. Residual pellet from cellular compartment fractionation, containing intact cells and organelles, was deprived of the former through centrifugation and subjected to Triton X-100 extraction in order to obtain soluble and insoluble fractions which were immunoprecipitated with rabbit anti TUCAP-I. Following electrophoresis of samples, the nitrocellulose was blotted with mouse anti-TUC AP- 1.

Immunofluorescence analyses. MM2 cells were seeded on cover glass placed in 60-mm Petri dishes. Cells were fixed with 2% paraformaldehyde and permeabilized (Triton X-100 (0.1%) or 24hous. For TUCAP 1 and Rab5 double staining cells were labeled with mouse anti-TUC AP-I serum and rabbit anti-Rab5 (SantaCruz) and respectively revealed with Alexa Fluor 488-conjugated anti-mouse IgG and anti-rabbit Alexa Fluor 594- conjugated IgG (Molecular Probes). For TUCAP-I and Lamp-1 detection cells were labeled with rabbit anti-TUCAP-1 pAb and mouse anti Lamp-1 Mab, (BD Pharmingen) respectively, stained with Alexa Fluor 594-conjugated anti-rabbit IgG and Alexa Fluor 488- conjugated anti-mouse IgG. TUCAP-I and mitochondria were detected by staining TUCAP-I with anti-TUCAP-1 mouse pAb and labeled with Alexa Fluor 488-conjugated anti-mouse IgG, while mitochondria were labeled with Mithotracker Red (Invitrogen). After washings, all samples were mounted with glycerohPBS (2:1) and observed with a Leica DM 2500 fluorescence microscope. Images were recorded with a Spot Insight digital camera (Delta Sistemi) equipped with IAS 8.2 system of image analysis (Delta Sistemi).

MM2 whole cell lysates were immunoprecipitated with anti-TUCAP-1 antibodies and various subcellular fractions were separated and analyzed by Western blot. The results revealed that TUCAP-I was mainly recovered in fractions enriched for cellular organelles, while undetectable in sytosolic and plasma membrane fractions (Fig. 5A). In order to identify subcellular localization of TUCAP-I, MM2 cells were double stained for TUCAP-I, and either for the early endosomal markers Rab5, or for the component of late endosomes and lysosomes Lamp-1, or the mitochondrial marker Mitotracker TM.

Fluorescence microscopy analysis showed that TUCAP-I co localized with both Rab5 (Fig. 5B) and EEA-I (Fig. 6 and Fig. 7B), while it did not co-localize with either Lamp-1 (Fig. 5C), Mitotracker TM (Fig. 5D) or Hoechst stained nuclei.

Moreover, Figure 7 shows the double staining fluorescence on same cells co-cultured with living lymphocytes. TUCAP-I is detectable exclusively on the surface of melanoma cells, while lymphocytes are completely unstained (Figure 7A). Again, TUCAP-I co-localizes with the primary endosome marker EEA-I (Figure 7B) confirming the expression of this protein on early endosomes.

EXAMPLE 4

Functional analysis of TUCAP-I

Tucap-1 silencing inhibits phagocytic behaviour

The role of TUCAP-1 protein in human metastatic melanoma cells was evaluated by inhibiting its expression through *Tucap-1* silencing.

The following StealthRRNAi duplexes (Invitrogen) were used for tucap-1 silencing:

gagugacguccagauccacugguuu (SEQ ID NO: 13), and
aaaccaguggaucuggacgucacuc (SEQ ID NO: 14), and annealed according to the manufacturer's instructions. As a negative control Stealth RNAi Negative control medium GC duplexes (Invitrogen) was used. Melanoma cells were transfected using Lipofectamine RNAiMAX reagent (Invitrogen) according to the manufacturer's instructions. Briefly the day before transfection, melanoma cells were seeded in six- well plates (IXIO⁵ per well), and after 24 hours, cells were transfected with 30pmol of siRNA per well. 48 hours after transfection, cells were analyzed for TUCAP-1 expression by FACS analysis.

Three different phagocytic metastatic cell lines were transfected with small interfering RNA to *Tucap-1* (Tucap-1 siRNA), or transfected with an irrelevant siRNA oligo (SC-siRNA). Fig.8 shows FACS analysis of TUCAP-1 expression on untransfected MM2 cells and Scrambled siRNA or TUCAP-1 siRNA transfected MM2 cells 48 hours after transfection. Similar results were obtained in scrambled and TUCAP-1 silenced MM3 cells (not shown). This confirmed an effective knockdown of TUCAP- 1.

To assess the phagocytic activity of these cells, we first measured the ability of untransfected, SC-siRNA transfected, or *Tucap-1* silenced melanoma cell lines to ingest stained yeast cells or living lymphocytes. 48 hours after transfection, SC-siRNA or TUCAP-1 siRNA transfected MM2 and MM3 melanoma cells were incubated at 37° C with FITC stained *Saccaromyces* yeasts FITC (1 :60), or 10 uMol dihydrorhodamine 123 (DHR123) (Molecular Probes) stained living lymphocytes (1:10).

Phagocytosis/cannibalism was measured after 4 hours by washing away the excess lymphocytes or yeast cells with PBS and adding a PBS solution containing trypsin (1.5 g/L) EDTA (0.44g/L). After washings, melanoma cells were harvested and analyzed on a cytometer equipped with a 488-nm argon laser. At least 10,000 events were acquired and analyzed by a Macintosh computer using CellQuest software (Becton Dickinson).

Melanoma cells that appeared fluorescent in green were considered as phagocytic/cannibal. The results showed that the TUCAP-1 knocking-down markedly inhibited both the phagocytic and the cannibal activity of melanoma cells (Fig. 9A, 9B, Table 1), proving that TUCAP-1 plays a key role in the cannibal behavior of metastatic human melanomas and the protein thus can be used as a marker of malignancy.

Table 2. Role of TUCAP-1 in phagocytosis/cannibalism.

Phagocytic/Cannibal activity of scrambled siRNA transfected (SC-siRNA) and *Tucap-1* silenced (*Tucap-1* siRNA) MM2 and MM3 metastatic melanoma cells against FITC stained yeasts and DHR123 stained live lymphocytes. The phagocytic activity was expressed as % of phagocytic cells. Numbers are meanfcs.d. of 5 different experiments.

Yeasts Live lymphocytes

SC-siRNA *Tucap-1* siRNA SC-siRNA *Tucap-1* siRNA

MM2 37 ± 1 4 ± 2 45 ± 5 17 ± 9

MM3 43 ± 14 14 ± 12 40 ± 9 9 ± 7

EXAMPLE 5

TUCAP-1 has a role in regulating acidification of endosomal vesicles

Scrambled siRNA transfected and TUCAP-1 silenced MM2 and MM3 cells were stained with 1 µM LysoTracker probe (Molecular Probes) for 30 minutes at 37 °C and immediately

29 May 2014
2009207926

analyzed by a cytometer. Comparisons among different melanoma cell lines were conducted by CellQuest software using the median values of fluorescence intensity histograms.

Based on the result that TUCAP-1 localizes on Rab5 bearing endosomes (see EXAMPLE 3), we tested a hypothesis that TUCAP-1 protein may have a role in the pH regulation of phago/endosomal compartments of malignant tumor cells. To verify this hypothesis, control SC-RNAi and TUCAP-1 -siRNA transfected cells were stained with the acidotropic probe LysoTracker green and analyzed by flow symmetry. TUCAP-1 gene silencing induced appearance of less acidic vesicle within melanoma cells, as compared to SC-RNA transfected control cells (FIG 9C). These experiments support the hypothesis that TUCAP-1 has a role in regulating acidification of internal vesicles, such as early endosomes.

EXAMPLE 6

TUCAP-1 involvement in early phases of metastatic process

Ongoing experiments based on using TUCAP-1 overexpressing cells suggest that this protein is involved in tumor cell invasiveness during early phases of metastatic process. Cell invasion capability of these cells is assayed by using Matrigel invasion chambers (Becton-Dickenson, Bedford, MA, USA). Briefly, untransfected WM743 or GFP-Tagged full length TUCAP-1 WM743 melanoma cells (TWM) were resuspended in serum free medium and loaded into the top chamber, while in the bottom chamber was placed in medium added with 10 % FCS as a chemoattractant. Cells were incubated at 37°C in a humidified atmosphere and allowed to migrate through the chemotaxis chamber for 48 hours. After incubation, the cells remaining at the upper surface were completely removed using a cotton carrier. The migrated cells on the bottom of chemotaxis chamber were stained with crystal violet. Invading cells were counted microscopically (40x) in four different fields per filter. Figure 10 shows the lower side of transwell membrane, clearly indicating that the number of invading cells was significantly higher for TWM, with a mean of 25 cells for untransfected versus a mean of 132 cells for TUCAP 1 transfected TWM cells.

EXAMPLE 7

TUCAP-1 involvement in cisplatin resistance of melanoma cells

Several publications show the role of proteins involved in ion trafficking and the role of endo-lysosomal compartment in drug sequestering, inactivation and extrusion as

mechanisms of drug resistance. TUCAP 1 expression in early endosomes and its involvement in pH regulation of endosomal vesicles (as shown in above examples) led us to hypothesize a role for this protein in drug resistance of cancer cell. To prove this, MM2 melanoma cells, highly expressing TUCAP-I, were pretreated with Scrambled (SC-siRNA) or Tucap-1 si-RNA for 48 hours (as shown in the previous examples), and after transfection cells were treated with 2uM cisplatin. 48 hours after cisplatin induced cytotoxicity was evaluated by FACS analysis of early (annexin-V single positive) and late (PI/Annexin V double positive) apoptosis. Tucap-1 silencing markedly increased cytotoxic effects of cisplatin as compared to Scrambled-si-RNA treated WM743 cells that behaved as the untransfected control cells. With a mean of 63 % of live cells in control transfected cells versus a mean of 37 % in TUCAP-1 silenced cells.

This set of experiments proves that TUCAP-1 is involved in drug resistance of TUCAP-1 over expressing cells and that *tucap-1* silencing is a promising method to inhibit phagocytotic character of tumor cells.

- Based on the results shown in above examples, the present disclosure also teaches highly sensitive and specific methods for detection of melanoma and several other tumors characterized by a described phagocytic behaviour i.e. breast cancer, lung carcinoma, bladder cancer, medulloblastoma, and gastric adenocarcinoma. Moreover, this disclosure teaches means to distinguish malignant from benign cancer lesions by showing that TUCAP-1 is exclusively expressed in malignant tumors. Methods for cancer detection comprise evaluation of a biological sample from a putative cancer lesion, typically by in situ hybridization, rt-PC, or immunoenzymatic methods.

EXAMPLE 8

Polyclonal and Monoclonal Antibodies to TUCAP-I

- Based on the examples above, polyclonal (pAb) and monoclonal antibodies (mAb) specific for TUCAP-I would be useful for various purposes, including for example diagnostics to determine malignancy of a tumor, and treatment of cancer.

In order to produce polyclonal antibodies, cDNA from MMI cells was cloned in bacterial expression vectors to obtain TUCAP-I aminoacids 18-282 fused to a 6-Histidine N-

terminal tag (SEQ ID NO: 11). Purified recombinant peptide was used to produce anti-TUCAP-1 antibodies in mice. The anti-TUCAP-1 antibodies recognized immunogen, GFP-tagged full length protein as positive control as well as endogenous TUCAP-I protein.

Polyclonal antibodies were also generated by immunizing rabbit, goat and donkey with a purified peptide fragment having an amino acid sequence according to SEQ ID NO: 12 . The antibodies generated were able to recognize human TUCAP-I protein by binding to a peptide fragment that consists of amino acids 221-235 of SEQ ID NO: 1. Polyclonal antibodies are also obtained by immunizing goat and donkey.

In order to produce monoclonal antibodies, mice were immunized with a peptide fragment having amino acid sequence according to SEQ ID NO: 11 or SEQ ID NO: 12 or a fragment thereof. Alternatively mice are immunized with peptide fragment having amino acid sequence according to SEQ ID NO: 15 . Selected hybridoma clones were generated by using spleen cells of selected mice. Briefly B-cells deriving from spleen of immunized mice were fused with a myeloma tumor cell line specifically selected for hybridoma production. The deriving fused (hybrid) cells that can grow indefinitely in culture with consequent production large amounts of the desired antibodies. Hybridoma production was performed according to standard protocols. After screening the selected hybridomas, the hybridomas are cloned and grown to large-scale for antibody production. Various positive hybridomas are selected for different uses, for example: a) laboratory experimental uses (Western Blot, immuno- precipitation, FACS analysis, immunofluorescence and immunohisto- and immunocyto-chemical analysis of human tissues and cultured cells); b) preclinical and clinical studies, c) tumor diagnosis and prognosis tools, such as detection kit. The monoclonal antibodies produced bind to conformational or linear epitopes of TUCAP-I protein amino acids 18-282 of SEQ ID NO: 1 or peptide fragment consisting of amino acids 221-235 or consisting of amino acids 303-352 of SEQ ID NO: 1. The antibodies also bind to TUCAP-I protein of mouse, rat, cat, dog, and sheep origin.

The examples above disclose particular embodiments of the invention in detail. However, this has been done by way of example and for the purposes of illustration only. The examples are not intended to limit the scope of the appended claims, which define the invention.

REFERENCES

29 May 2014
2009207926

Bukrinskaya A, Brichacek B, Mann A, Stevenson M. Establishment of a functional human immunodeficiency virus type 1 (HIV-1) reverse transcription complex involves the cytoskeleton. *J Exp Med.* 1998;188:2113-2125.

Caruso RA, Muda AO, Bersiga A, Rigoli L, Inferrera C. Morphological evidence of
5 neutrophil-tumor cell phagocytosis (cannibalism) in human gastric adenocarcinomas. *Ultrastruct Pathol* 2002; 26:315-21.

Chou, K.C. (2005). "Using amphiphilic pseudo amino acid composition to predict enzyme subfamily classes". *Bioinformatics*, 21, 10-19.

DeSimone PA, East R, Powell RD. Phagocytic tumor cell activity in oat cell carcinoma of
10 the 6y7y- j - -- ol 1980;l 1 :535-9.

Fujii M, Ishii Y, Wakabayashi T, et al. Cytologic diagnosis of male breast cancer with nipple discharge. A case report. *Acta Cytol* 1986;30:2.

Gasteiger E., Gattiker A., Hoogland C, Ivanyi I., Appel R.D., Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis *Nucleic Acids Res.* 31
15 :3784-3788(2003).

Kojima S, Sekine H, Fukui I, Ohshima H. Clinical significance of "cannibalism" in urinary cytology of bladder cancer. *Acta Cytol* 1998;42: 1365-9.

Kumar PV, Hosseinzadeh M, Bedayat GR. Cytologic findings of medulloblastoma in crush smears. *Acta Cytol* 2001;45:542-6.

20 Lugini L, Lozupone F, Matarrese P, Funaro C, Luciani F, Malorni W, Rivoltini L, Castelli C, Tinari A, Piris A, Parmiani G, Fais S. Potent phagocytic activity discriminates metastatic and primary human malignant melanomas: a key role of ezrin. *Lab Invest.* 2003 Nov;83(11):1555-67.

Lugini L, Matarrese P, Tinari A, Lozupone F, Federici C, Iessi E, Gentile M, Luciani F,
25 Parmiani G, Rivoltini L, Malorni W, Fais S. Cannibalism of live lymphocytes by human metastatic but not primary melanoma cells. *Cancer Res.* 2006 Apr 1 ;66(7):3629-38.

29 May 2014
2009207926

B Rost, G Yachdav and J Liu (2004) Server. Nucleic Acids Research 32(Web Server issue):W321-W326.

B Rost (1996) Methods in Enzymology, 266:525-539.

- 5 Shen, H. B. and Chou, K. C (2007) "Hum-mPLoc: an ensemble classifier for large-scale human protein subcellular location prediction by incorporating samples with multiple sites", Biochemical and Biophysical Research Communications. 355(4):1006-11.

Sloane BF, Dunn JR, Honn KV. Lysosomal cathepsin B: correlation with metastatic potential. Science 1981; 212:1151-3.

Steinhaus J. Ueber carcinoma-einschlusse. Virchows Arch 1891; 126:533-5.

- 10 Stroebe H. Zur Kenntniss verschiedener cellularer Vorgange und Erscheinungen in Geschwulsten. Beitrage Pathol 1892; 11 : 1.

Shen, H. B. and Chou, K. C. (2006). "Ensemble classifier for protein folding pattern recognition". Bioinformatics, Jul 15;22(14):1717-22.

The term “comprising” as used in this specification means “consisting at least in part of”.

- 15 When interpreting each statement in this specification that includes the term “comprising”, features other than that or those prefaced by the term may also be present. Related terms such as “comprise” and “comprises” are to be interpreted in the same manner.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

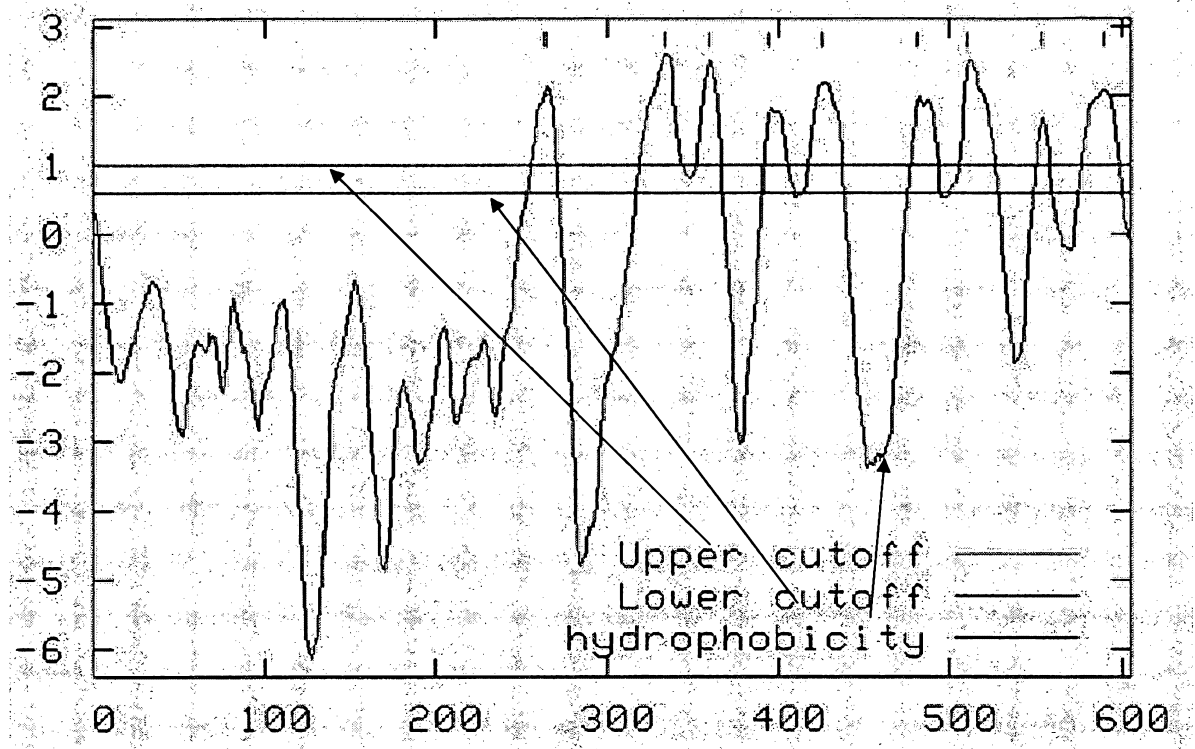
1. A marker for tumor malignancy, said marker consisting of amino acid sequence according to SEQ ID NO:11; SEQ ID NO:12 or SEQ ID NO:15.
2. The marker of claim 1, wherein the tumor is a melanoma tumor.
3. An isolated antibody, generated by immunizing mice, rabbit, goat or donkey with a peptide fragment having an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12 and SEQ NO: 15, wherein the antibody is capable of recognizing human TUCAP-1 protein by binding to a peptide fragment selected from the group consisting of amino acids 18-282, 221-235 and 303-352 of SEQ ID NO:1.
4. The antibody of claim 3 , or fragment thereof, wherein the antibody is a monoclonal antibody and belongs to IgG isotype IgG2A, or IgG isotype IgG1.
5. Monoclonal antibodies against human TUCAP-1 protein, said antibodies having a tumor growth blocking activity or metastasis blocking activity, said antibodies being generated by immunizing mice with a peptide fragment selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:15, and said antibodies recognizing human TUCAP-1 protein by binding to conformational or linear epitopes locating between aa 18 to 282, or 221-235 or 303-352 of SEQ ID NO:1.
6. Hybridomas obtained by using spleen cells of mice immunized with a peptide fragment having an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12 and SEQ NO: 15, and producing monoclonal antibodies, or fragments or humanized forms of such monoclonal antibodies or single chain forms of the fragments capable of binding to conformational or linear epitopes of the human TUCAP-1, said epitopes locating between amino acids 18- 282, or 221-235 or 303-352 of SEQ ID NO:1.
7. A kit for detecting malignant tumors *in vitro*, said kit comprising antibodies capable of recognizing human TUCAP-1 protein by binding to a peptide fragment selected from the group consisting of amino acids 18-282, 221-235 and 303-352 of SEQ ID NO:1 ; and a titred positive control comprising a peptide fragment having an amino acid sequence

selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 15, wherein the antibody is used to detect an oncogenic protein according to SEQ ID NO: 1 from tissues and body fluids of patients with tumors.

8. The kit of claim 7, wherein the tumor is a melanoma tumor.
9. A method to distinguish metastatic tumors *in vitro* from non-metastatic tumors, said method comprising a step of defining presence or absence of expression of SEQ ID NO: 1 in tumor cell, wherein presence of the expression indicates metastatic character of the cell.
10. The method of claim 9, wherein the tumor is a melanoma tumor.
11. A method of claim 9, wherein the presence or absence of SEQ ID NO:1 includes recognition of TUCAP-1 protein by an antibody capable of recognizing human TUCAP-1 protein by binding to a peptide fragment selected from the group consisting of amino acids 18-282, 221-235 and 303-352 of SEQ ID NO:1.
12. A method to follow up metastatic activity of a tumor, said method comprising a step of defining presence or absence of expression of SEQ ID NO: 1 in tumor cell, wherein presence of expression indicates metastatic activity.
13. The method of claim 12, wherein the tumor is melanoma tumor.
14. The method of claim 12, wherein the presence or absence of SEQ ID NO:1 includes recognition of TUCAP-1 protein by antibody capable of recognizing human TUCAP-1 protein by binding to a peptide fragment selected from the group consisting of amino acids 18-282, 221-235 and 303-352 of SEQ ID NO:1.
15. A method of inhibiting phagocytotic character of tumor cells, said method comprising a step to inhibit expression of SEQ ID NO: 1.
16. The method of claim 15, wherein the expression is inhibited by silencing expression of *Tucap-1* gene.

17. The method of claim 15, wherein tumor cells are transfected with an expression vector comprising an isolated nucleic acid sequence that is substantially complimentary to SEQ ID NO: 2, or part of it, or complementary to TUCAP-1 mRNA or TUCAP-1 specific regulating factors.
18. A method to treat metastatic tumors, said method comprising a step of administering to a patient a therapeutically effective amount of a composition comprising a TUCAP-1 binding agent conjugated to a chemotherapeutic drug.
19. Use of a TUCAP-1 binding agent conjugated to a chemotherapeutic drug for the manufacture of a medicament for treating metastatic tumors.
20. A marker as claimed in claim 1 or claim 2; an isolated antibody as claimed in claim 3 or claim 4; monoclonal antibodies as claimed in claim 5; hybridomas as claimed in claim 6; a kit as claimed in claim 7 or claim 8; a method as claimed in any one of claims 9 to 11, to distinguish metastatic tumors *in vitro* from non-metastatic tumors; a method as claimed in any one of claims 12 to 14 to follow up metastatic activity of a tumor; a method as claimed in any one of claims 15 to 17 of inhibiting phagocytotic character of tumor cells; a method as claimed in claim 18 to treat metastatic tumors; or a use as claimed in claim 19 of a TUCAP-1 binding agent; substantially as herein described with reference to any example thereof and with or without reference to the accompanying drawings.

1 / 15

A**B**

unknown

Structure no. 1

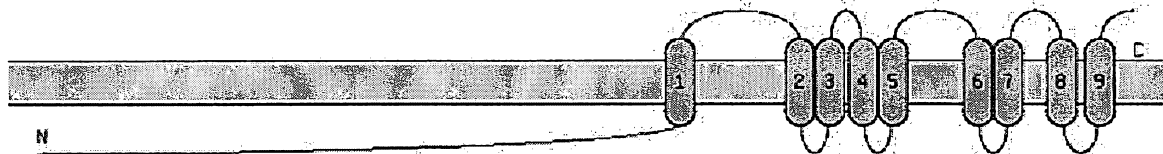
Segments included: 1 2 3 4 5 6 7 8 9

□ Segment Putative

■ Segment Certain

CYTOPLASM

LI = 49 LI = 14 LI = 35 LI = 22 = 14
KR = 6 KR = 6 KR = 6 KR = 2 KR = 3



LI = 264
KR = 29

LI = 11 LI = 11 LI = 8 LI = 15
KR = 1 KR = 1 KR = 0 KR = 1

LI: Loop length
KR: Number of Lys and Arg

EXTRACELLULAR

Figure 1

2 / 15

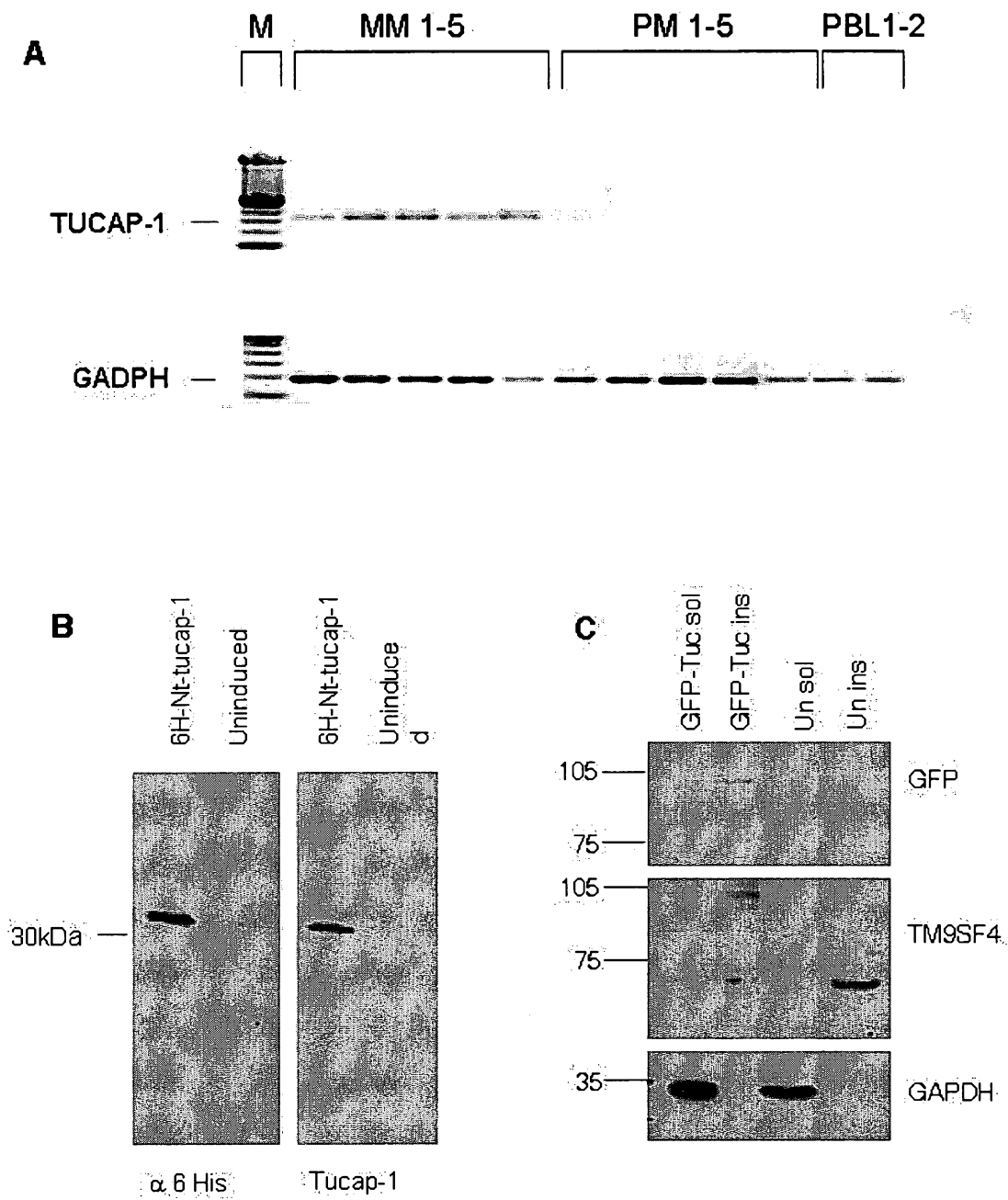


Figure 2

3 / 10

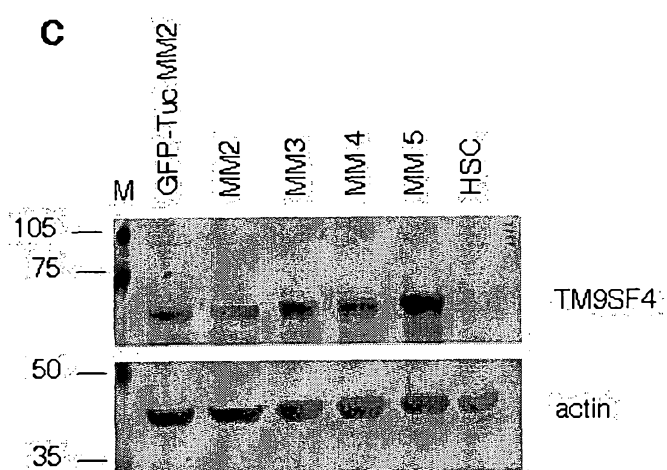


Figure 3

4 / 15

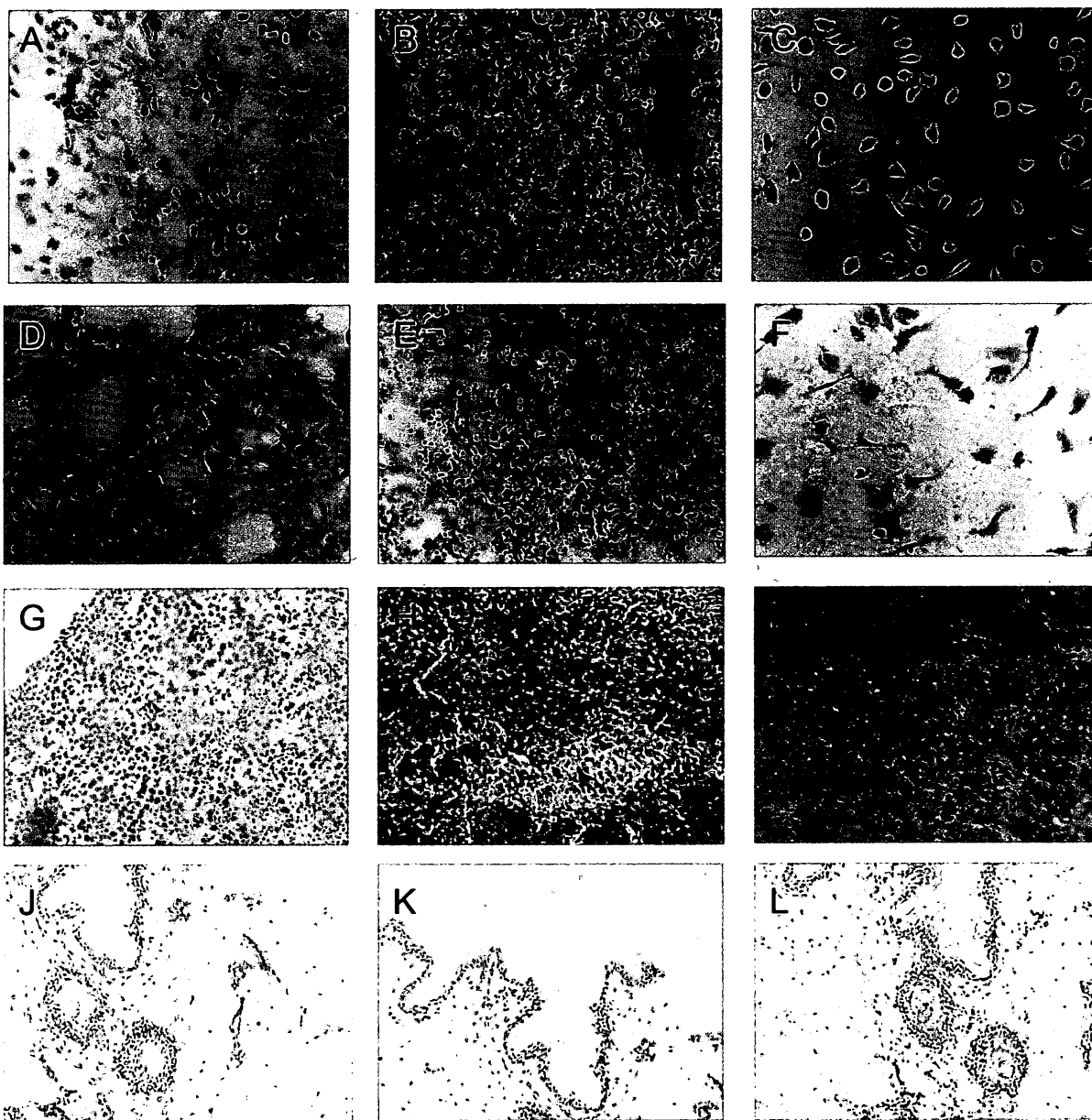


Figure 4

5 / 15

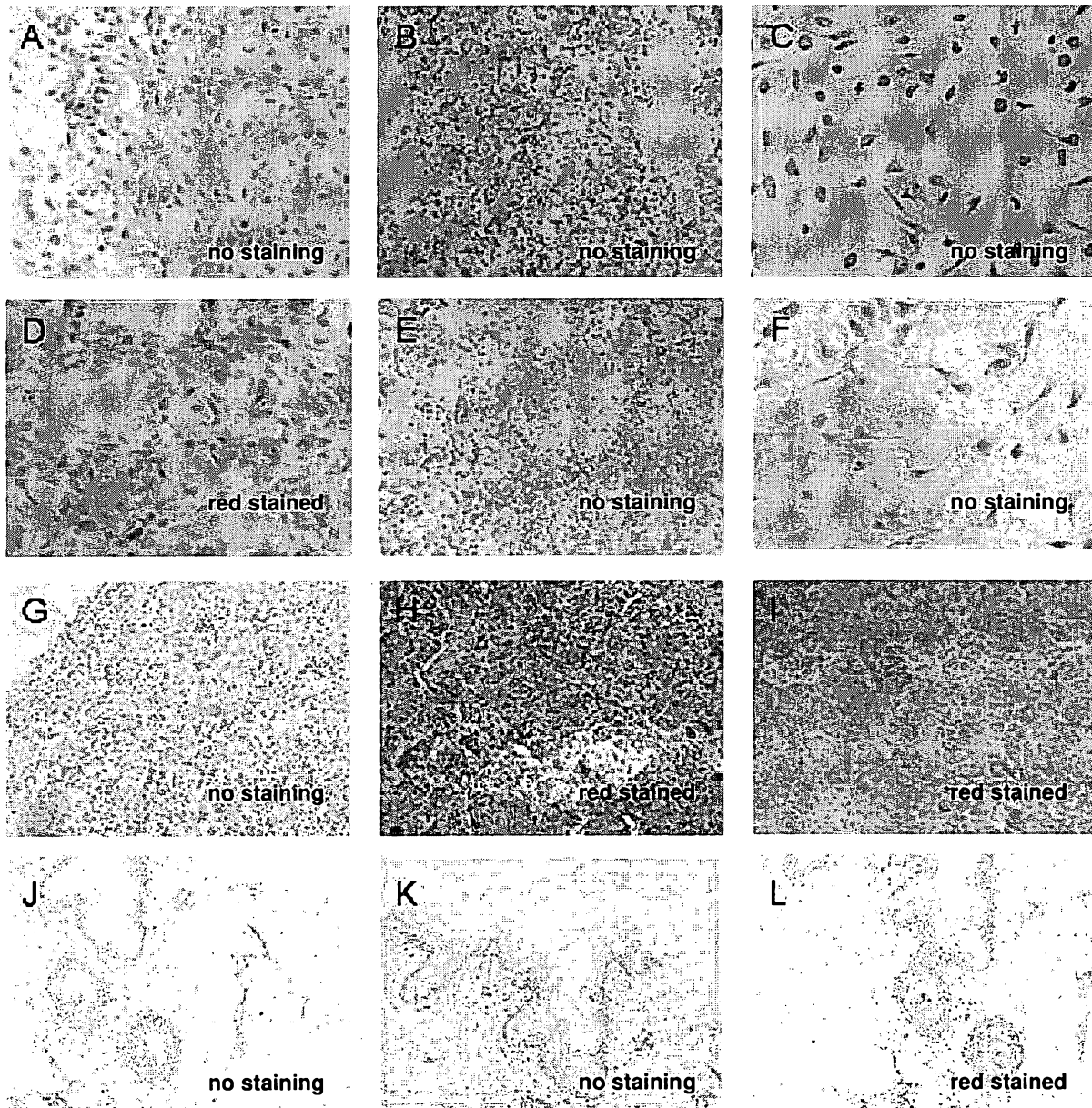


Figure 4A

6 / 15

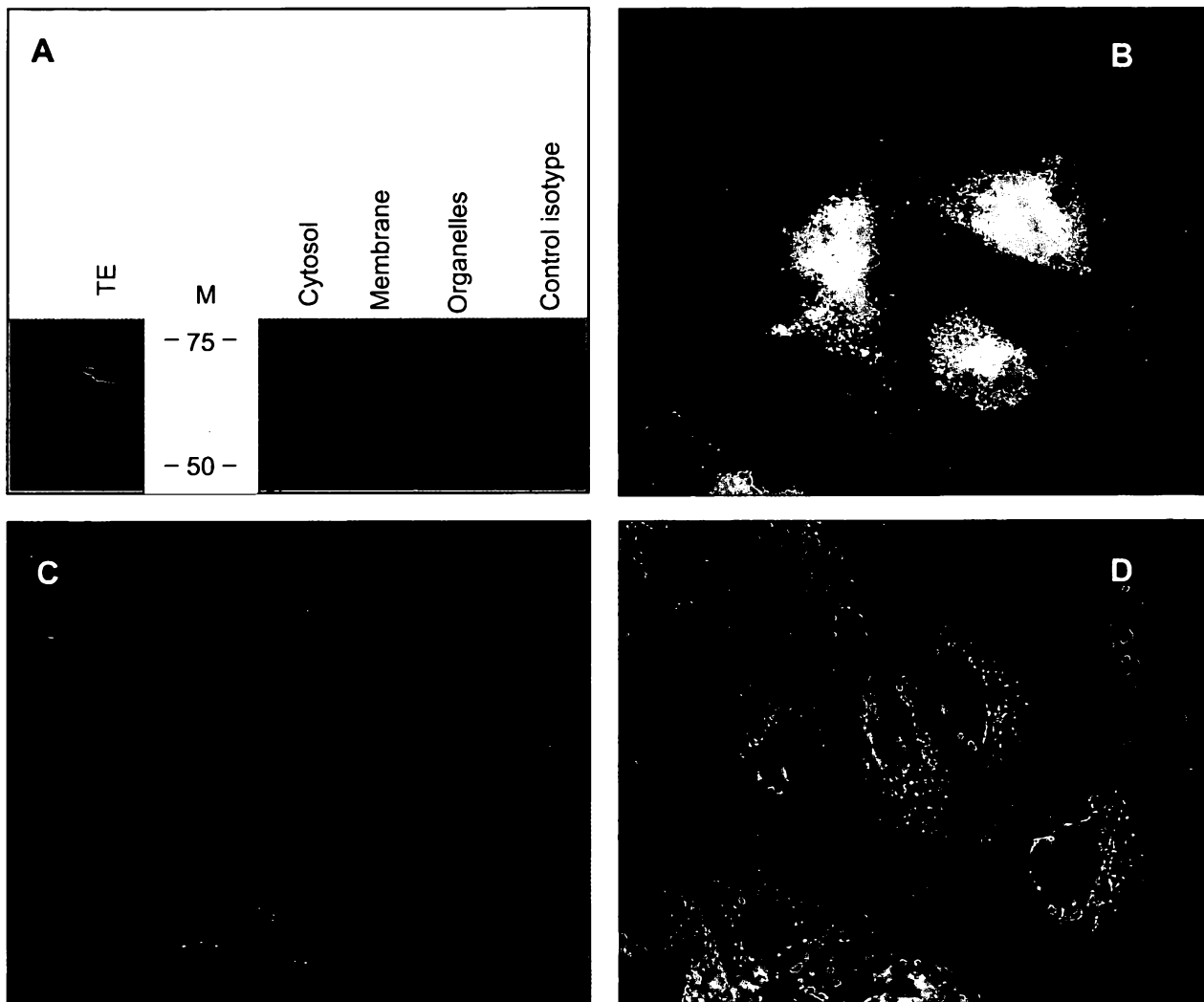


Figure 5

7 / 10

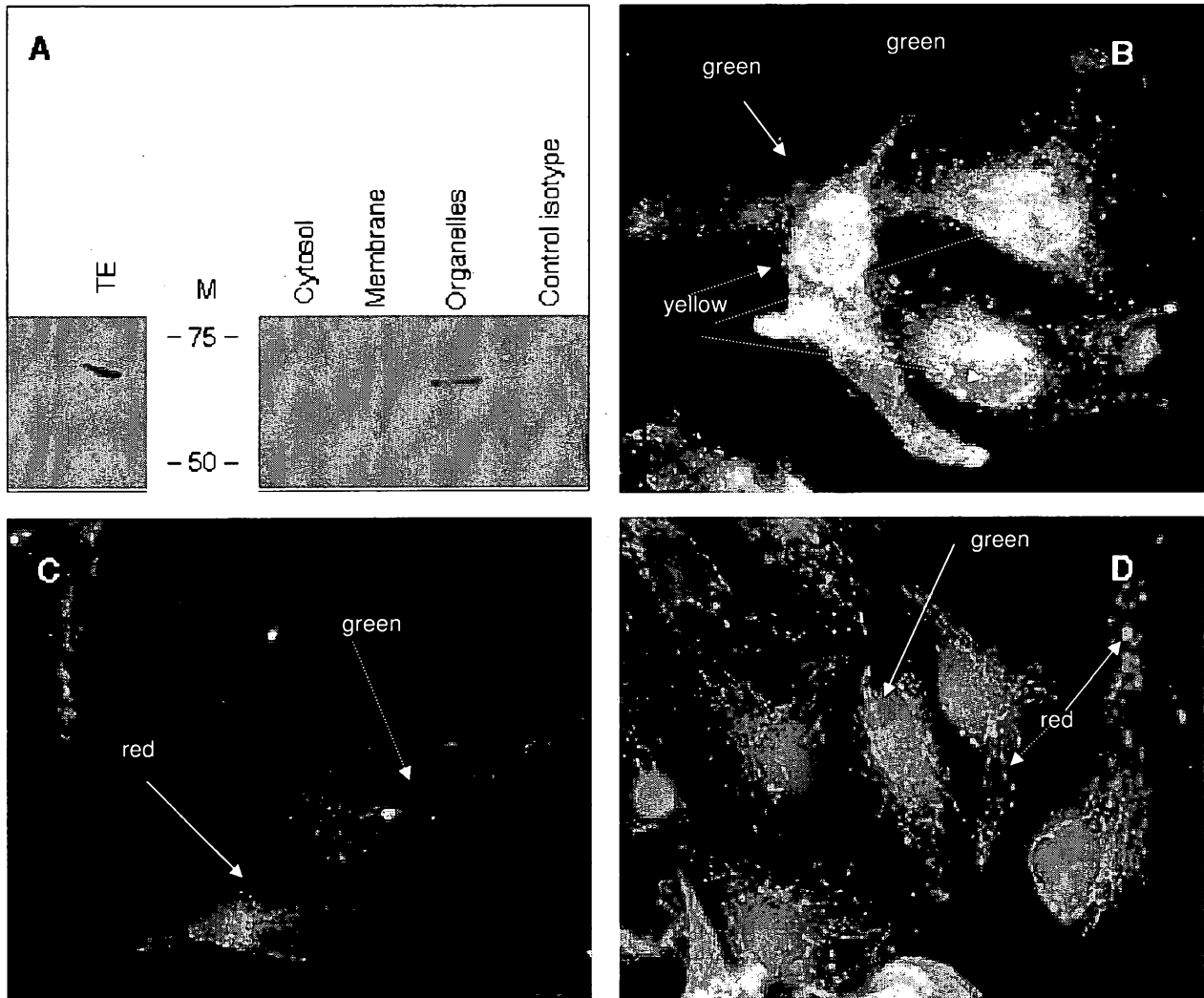


Figure 5A

8 / 15

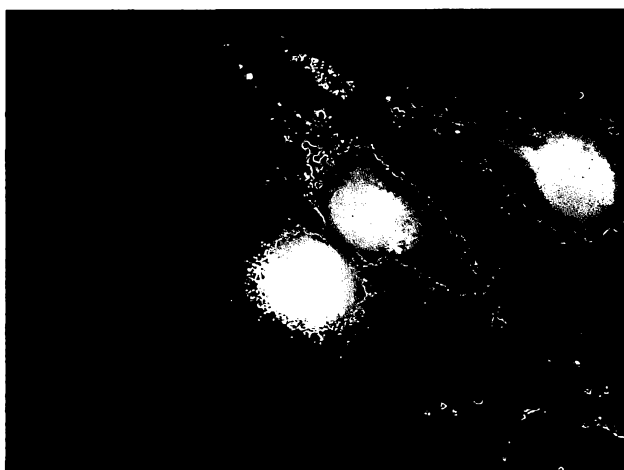


Figure 6

9 / 15

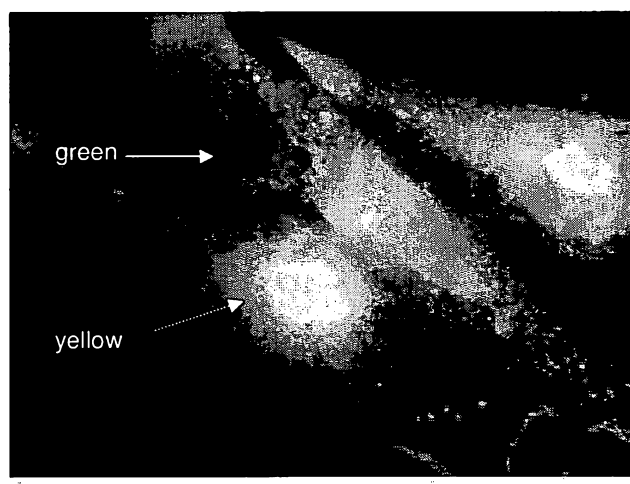


Figure 6A

10 / 15

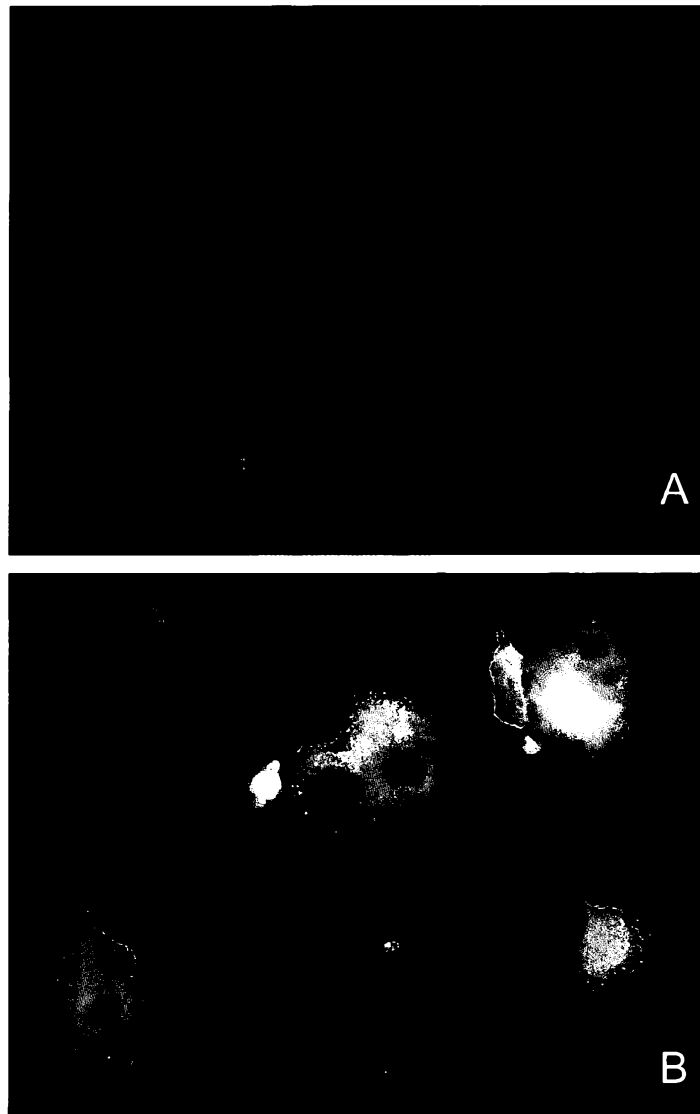


Figure 7

11 / 15

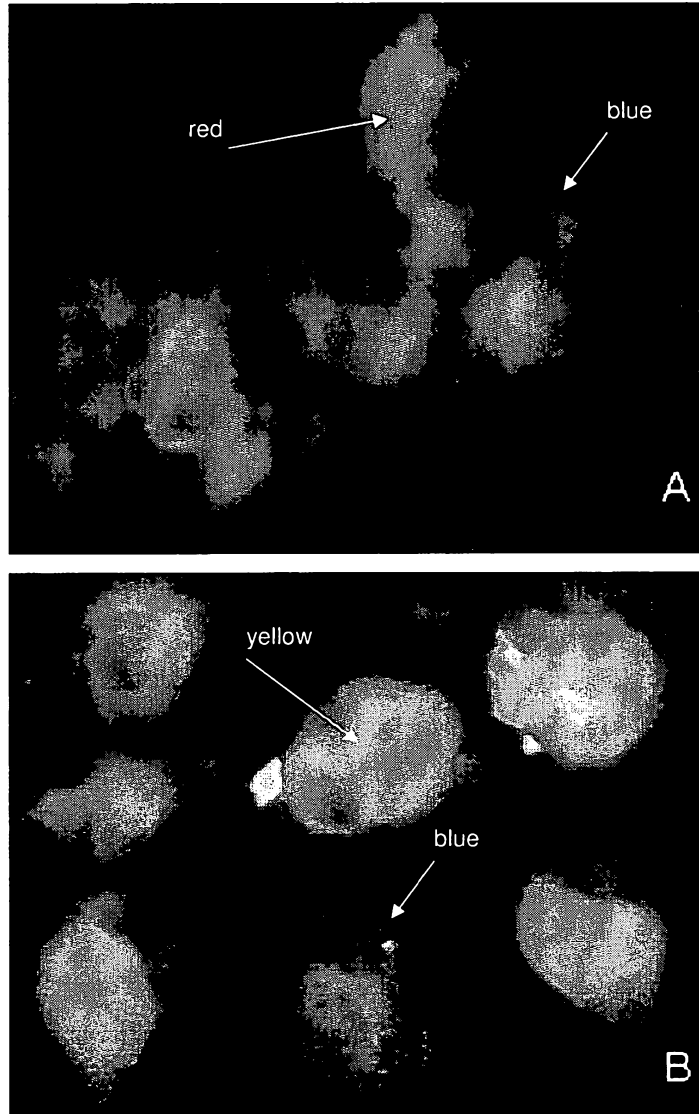


Figure 7A

12 / 15

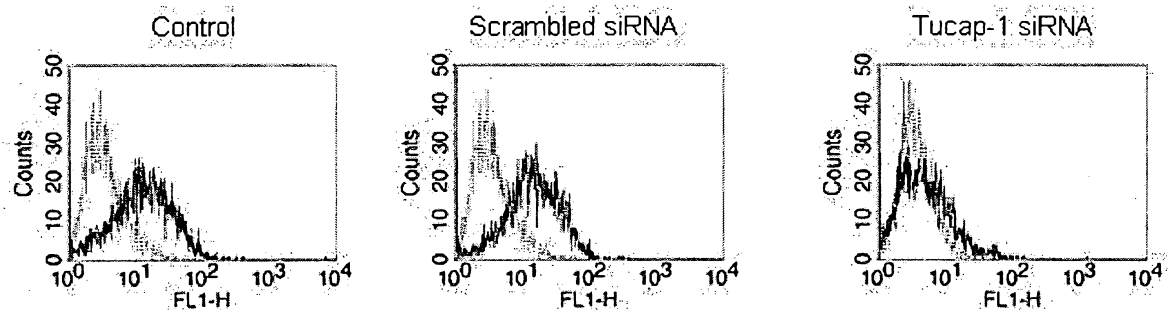


Figure 8

13 / 15

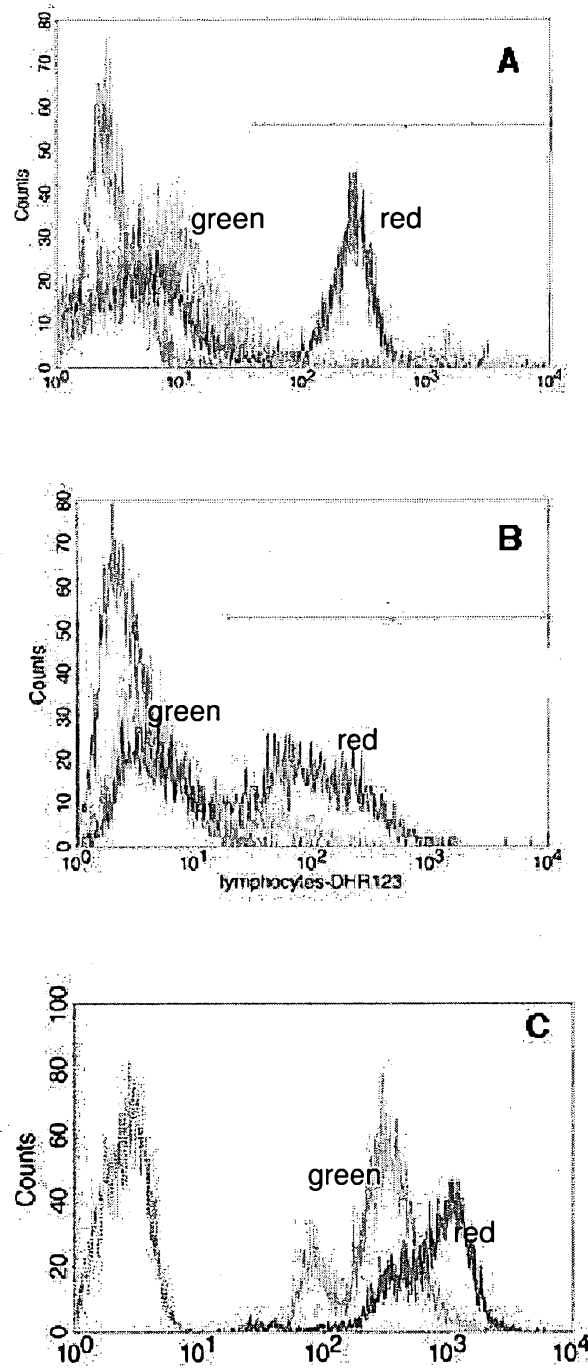
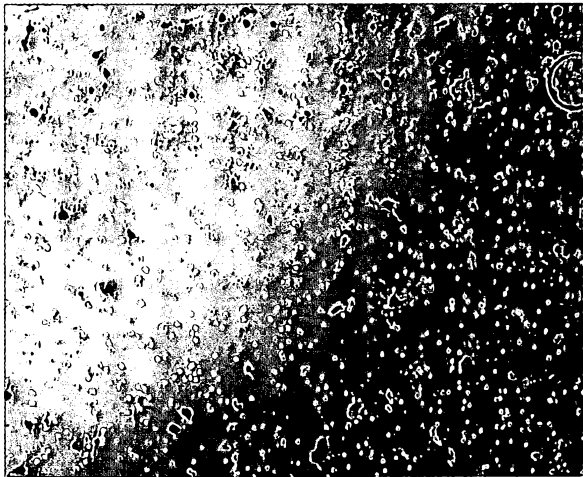
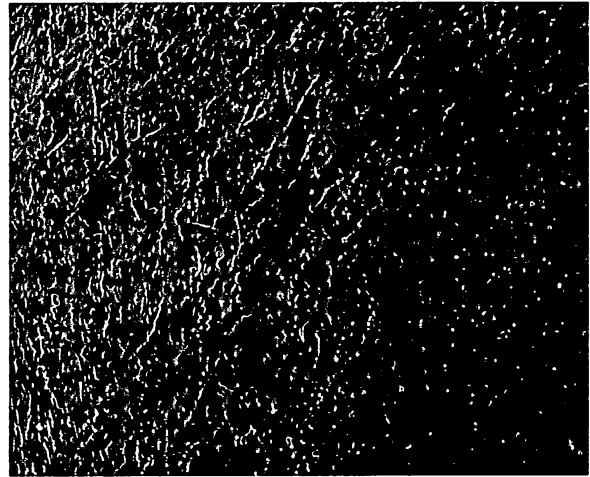


Figure 9

14 / 15



WM743



TWM

Figure 10

15 / 15

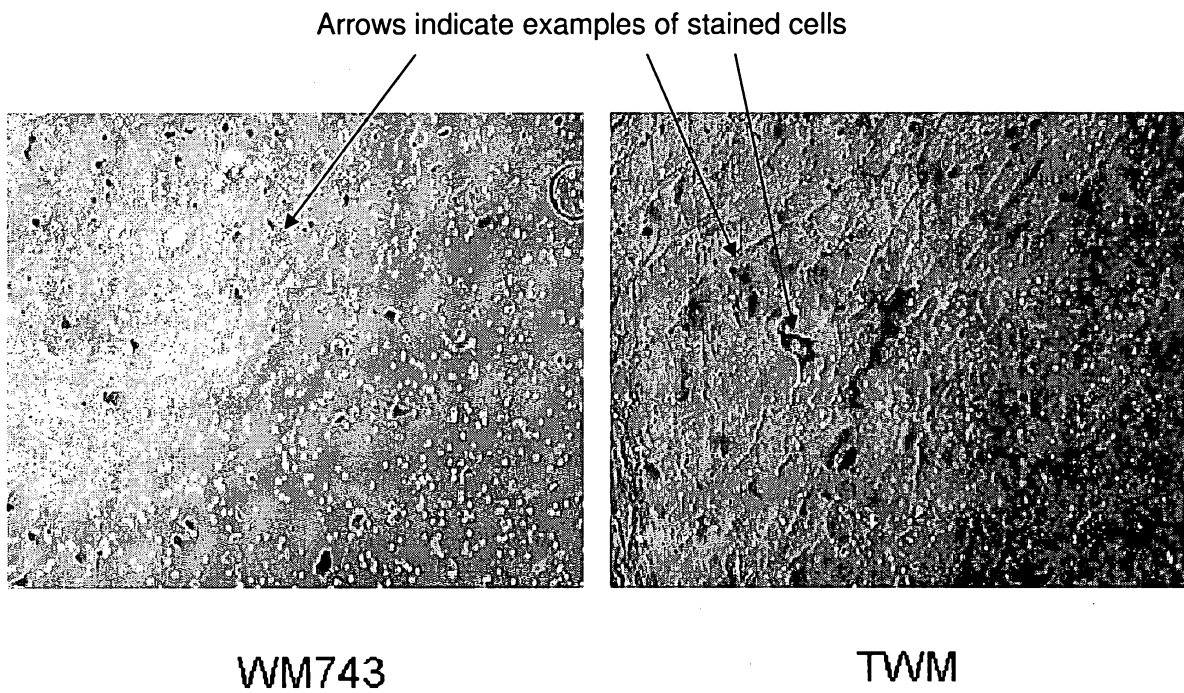


Figure 10A

Tucap sequence listing.ST25.txt
SEQUENCE LISTING

<110> HANSABIOMED OÜ

<120> A new metastatic human tumor associated molecule, methods to detect both activated gene and protein and to interfere with gene expression

<130> TUCAP

<150> 61/062,453

<151> 2008-02-25

<160> 15

<170> PatentIn version 3.3

<210> 1

<211> 642

<212> PRT

<213> Homo sapiens

<400> 1

Met Ala Thr Ala Met Asp Trp Leu Pro Trp Ser Leu Leu Leu Phe Ser
1 5 10 15

Leu Met Cys Glu Thr Ser Ala Phe Tyr Val Pro Gly Val Ala Pro Ile
20 25 30

Asn Phe His Gln Asn Asp Pro Val Glu Ile Lys Ala Val Lys Leu Thr
35 40 45

Ser Ser Arg Thr Gln Leu Pro Tyr Glu Tyr Tyr Ser Leu Pro Phe Cys
50 55 60

Gln Pro Ser Lys Ile Thr Tyr Lys Ala Glu Asn Leu Gly Glu Val Leu
65 70 75 80

Arg Gly Asp Arg Ile Val Asn Thr Pro Phe Gln Val Leu Met Asn Ser
85 90 95

Glu Lys Lys Cys Glu Val Leu Cys Ser Gln Ser Asn Lys Pro Val Thr
100 105 110

Leu Thr Val Glu Gln Ser Arg Leu Val Ala Glu Arg Ile Thr Glu Asp
115 120 125

Tyr Tyr Val His Leu Ile Ala Asp Asn Leu Pro Val Ala Thr Arg Leu
130 135 140

Glu Leu Tyr Ser Asn Arg Asp Ser Asp Asp Lys Lys Lys Glu Lys Asp
145 150 155 160

Val Gln Phe Glu His Gly Tyr Arg Leu Gly Phe Thr Asp Val Asn Lys
165 170 175

Tucap sequence listing.ST25.txt

Ile Tyr Leu His Asn His Leu Ser Phe Ile Leu Tyr Tyr His Arg Glu
 180 185 190

Asp Met Glu Glu Asp Gln Glu His Thr Tyr Arg Val Val Arg Phe Glu
 195 200 205

Val Ile Pro Gln Ser Ile Arg Leu Glu Asp Leu Lys Ala Asp Glu Lys
 210 215 220

Ser Ser Cys Thr Leu Pro Glu Gly Thr Asn Ser Ser Pro Gln Glu Ile
 225 230 235 240

Asp Pro Thr Lys Glu Asn Gln Leu Tyr Phe Thr Tyr Ser Val His Trp
 245 250 255

Glu Glu Ser Asp Ile Lys Trp Ala Ser Arg Trp Asp Thr Tyr Leu Thr
 260 265 270

Met Ser Asp Val Gln Ile His Trp Phe Ser Ile Ile Asn Ser Val Val
 275 280 285

Val Val Phe Phe Leu Ser Gly Ile Leu Ser Met Ile Ile Ile Arg Thr
 290 295 300

Leu Arg Lys Asp Ile Ala Asn Tyr Asn Lys Glu Asp Asp Ile Glu Asp
 305 310 315 320

Thr Met Glu Glu Ser Gly Trp Lys Leu Val His Gly Asp Val Phe Arg
 325 330 335

Pro Pro Gln Tyr Pro Met Ile Leu Ser Ser Leu Leu Gly Ser Gly Ile
 340 345 350

Gln Leu Phe Cys Met Ile Leu Ile Val Ile Phe Val Ala Met Leu Gly
 355 360 365

Met Leu Ser Pro Ser Ser Arg Gly Ala Leu Met Thr Thr Ala Cys Phe
 370 375 380

Leu Phe Met Phe Met Gly Val Phe Gly Gly Phe Ser Ala Gly Arg Leu
 385 390 395 400

Tyr Arg Thr Leu Lys Gly His Arg Trp Lys Lys Gly Ala Phe Cys Thr
 405 410 415

Ala Thr Leu Tyr Pro Gly Val Val Phe Gly Ile Cys Phe Val Leu Asn
 420 425 430

Cys Phe Ile Trp Gly Lys His Ser Ser Gly Ala Val Pro Phe Pro Thr
 435 440 445

Tucap sequence listing.ST25.txt

Met Val Ala Leu Leu Cys Met Trp Phe Gly Ile Ser Leu Pro Leu Val
 450 455 460

Tyr Leu Gly Tyr Tyr Phe Gly Phe Arg Lys Gln Pro Tyr Asp Asn Pro
 465 470 475 480

Val Arg Thr Asn Gln Ile Pro Arg Gln Ile Pro Glu Gln Arg Trp Tyr
 485 490 495

Met Asn Arg Phe Val Gly Ile Leu Met Ala Gly Ile Leu Pro Phe Gly
 500 505 510

Ala Met Phe Ile Glu Leu Phe Phe Ile Phe Ser Ala Ile Trp Glu Asn
 515 520 525

Gln Phe Tyr Tyr Leu Phe Gly Phe Leu Phe Leu Val Phe Ile Ile Leu
 530 535 540

Val Val Ser Cys Ser Gln Ile Ser Ile Val Met Val Tyr Phe Gln Leu
 545 550 555 560

Cys Ala Glu Asp Tyr Arg Trp Trp Trp Arg Asn Phe Leu Val Ser Gly
 565 570 575

Gly Ser Ala Phe Tyr Val Leu Val Tyr Ala Ile Phe Tyr Phe Val Asn
 580 585 590

Lys Leu Asp Ile Val Glu Phe Ile Pro Ser Leu Leu Tyr Phe Gly Tyr
 595 600 605

Thr Ala Leu Met Val Leu Ser Phe Trp Leu Leu Thr Gly Thr Ile Gly
 610 615 620

Phe Tyr Ala Ala Tyr Met Phe Val Arg Lys Ile Tyr Ala Ala Val Lys
 625 630 635 640

Ile Asp

<210> 2
 <211> 4028
 <212> DNA
 <213> Homo sapiens

<400> 2
 ccaagatggc gacggcgatg gtgagtgaag gagactccgg gagcgggagc tggagcgggg 60
 ccctccgggg tatcccagga tcttcagca ccccatgcct ggccctgagc cacctccggg 120
 acccctgact caggcctgag ggctacctct gactgggctt gtcttccccg aaatccacct 180
 ccctggccct gccctgcac tcaggcttgt gaaggcccg agttttgggg gaggcgccgt 240
 ttcggaggaa gacctcggct gctgccttcg ccggttccca ttctactttt ggtctccgcc 300

Tucap sequence listing.ST25.txt

cactgattgg ttgccgtggt ctttactgct tttctccctg atgtgtgaaa caagcgcctt	360
ctatgtgcct ggggtcgcgc ctatcaactt ccaccagaac gatcccgtag aaatcaaggc	420
tgtgaagctc accagctctc gaacccagct accttatgaa tactattcac tgcccttctg	480
ccagcccagc aagataacct acaaggcaga gaatctggga gaggtgctga gaggggaccg	540
gattgtcaac acccctttcc aggttctcat gaacagcgag aagaagtgtg aagttctgtg	600
cagccagtcc aacaagccag tgaccctgac agtggagcag agccgactcg tggccgagcg	660
gatcacagaa gactactacg tccacctcat tgctgacaac ctgcctgtgg ccacccggct	720
ggagctctac tccaaccgag acagcgatga caagaagaag gaaaaagatg tgcagtttga	780
acacggctac cggctcggct tcacagatgt caacaagatc tacctgcaca accacctctc	840
attcatcctt tactatcatc gggaggacat ggaagaggac caggagcaca cgtaccgtgt	900
cgtccgcttc gaggtgattc cccagagcat caggctggag gacctcaaag cagatgagaa	960
gagttcgtgc actctgcctg agggtagcaa ctctcgcgcc caagaaattg accccaccaa	1020
ggagaatcag ctgtacttca cctactctgt cactgggag gaaagtgata tcaaattggc	1080
ctctcgtgga gacacttacc tgaccatgag tgacgtccag atccactggt tttctatcat	1140
taactccgtt gttgtggtct tcttctgtc aggtatcctg agcatgatta tcattcggac	1200
cctccggaag gacattgcca actacaacaa ggaggatgac attgaagaca ccatggagga	1260
gtctgggtgg aagttggtgc acggcgacgt cttcaggccc cccagtagc ccatgatcct	1320
cagctccctg ctgggctcag gcattcagct gttctgtatg atcctcatcg tcatctttgt	1380
agccatgctt gggatgctgt cgcctccag ccggggagct ctcatgacca cagcctgctt	1440
cctcttcatg ttcattgggg tgtttgccg attttctgct ggccgtctgt accgcacttt	1500
aaaaggccat cgggtggaaga aaggagcctt ctgtacggca actctgtacc ctggtgtggt	1560
ttttggcatc tgcttcgtat tgaattgctt catttgggga aagcactcat caggagcggc	1620
gccctttccc accatggtgg ctctgctgtg catgtggttc gggatctccc tgcccctcgt	1680
ctacttgggc tactacttcg gcttccgaaa gcagccatat gacaaccctg tgcgaccaa	1740
ccagattccc cggcagatcc ccgagcagcg gtggtacatg aaccgatttg tgggcatcct	1800
catggctggg atcttgccct tcggcgccat gttcatcgag ctcttcttca tcttcagtgc	1860
tatctgggag aatcagttct attacctctt tggcttctg ttccttggtt tcatcatcct	1920
ggtggtatcc tgttcacaaa tcagcatcgt catggtgtac ttccagctgt gtgcagagga	1980
ttaccgctgg tgggtggagaa atttcctagt ctccgggggc tctgcattct acgtcctggt	2040
ttatgccatc ttttatttcg ttaacaagct ggacatcgtg gagttcatcc cctctctcct	2100
ctactttggc tacacggccc tcatggtctt gtccttctg ctgctaacgg gtaccatcgg	2160
cttctatgca gcctacatgt ttgttcgcaa gatctatgct gctgtgaaga tagactgatt	2220
ggagtggacc acggccaagc ctgctccgtc ctcggacagg aagccaccct gcgtggggga	2280
ctgcaggcac gcaaaataaa ataactcctg ctcgtttgga atgtaactcc tggcacagt	2340

Tucap sequence listing.ST25.txt

```

ttcctggatc ctggggctgc gtggggggcg ggagggcctg tagataatct tgcgtttttc 2400
gtcatcttat tccagttctg tgggggatga gtttttttgt gggttgcttt ttcttcagtg 2460
ctaagaaagt tccctccaac aggaactctc tgacctgttt attcaggtgt atttctggtt 2520
tggatttttt tttccttctt tgttttaaca aatggatcca ggatggataa atccaccgag 2580
ataagggttt tggtcactgt ctccacctca gttcctcagg gctgttggcc accctatgac 2640
taactggaag aggacacgcc agagcttcag tgaggtttcc gagcctctcc ctgcccattc 2700
tcaccactga ggccacgaca aagcacagct ccagctcgga cagcaccctc agtgccagcc 2760
agcctctgcc agacctctct ttcctctctc tccccagcct cctccagggc tgcccaaggc 2820
agggtttcca gccaggcctc ggggtcatct tttcaccagg agcaaacca agtcttagtt 2880
gctacaagaa aatcccttg aagtactggg ggccaggttc cccagacagc aggaattgcc 2940
cctgttcaga gcagccggag tttgctggac cacaaggaag aagagaagag acttgcatg 3000
aactgttttt gtgccaagaa accctggacc tggggccaag tatttcccaa gccaaagcatc 3060
cacttgctct tgtctgggaa gggatggcca aggccgctag ggtccttacc cctcaggatc 3120
actccccagc cttttctca ggaggtaccg ctctccaagg tgtgctagca gtgggccctg 3180
cccaacttca ggcagaacag ggaggcccag agattacaga tccccctctg taagtggcca 3240
ggcattctct ccctgccctc tctggcctct ggggtcatac tcacttcttt agccagcccc 3300
atcccccca cccacacct gagttcttgc ctctctcttt tggggacacc caaaacactg 3360
cttgtagaaa ggaagatgga aggtaagttc tgtcgttctt tccccaatcc ccaggaatgg 3420
acaagaagcc aacttagaaa gaagggctc acgtggctgg cctggctcct ccgtagaccc 3480
ctgttctttt caacctctgc ccaccctgc atgtcatcac aaacatttgc tcttaagtta 3540
caagagacca catccacca gggattaggg ttcaagtagc agctgctaac cttgcacca 3600
gcccttgtag gactcccaac acaagacaaa gtcaggatg ctggtgatgc taggaagatg 3660
tccctcccct cactgcccc cattctcca gtggctctac cagcctcacc catcaaacca 3720
gtgaatttct caatcttgcc tcacagtgc tgcagcgcca agcggcatcc accaagcatc 3780
aagttggaga aaaggggaacc caagcagtag agagcgatat tggagtcttt tgttcattca 3840
aatcttgga tttttttttt ccctaagaga ttctcttttt agggggaatg ggaaacggac 3900
acctcataaa gggttcaaag atcatcaatt tttctgactt tttaaatcat tatcattatt 3960
atttttaatt aaaaaaatgc ctgtatgcct ttttttggtc ggattgtaaa taaatatacc 4020
attgtcct 4028

```

<210> 3
 <211> 20
 <212> DNA
 <213> artificial

<220>
 <223> chemically synthesized sequence

Tucap sequence listing.ST25.txt

<220>
 <221> misc_feature
 <222> (1)..(20)
 <400> 3
 tgtgtgaaac aagcgccttc 20
 <210> 4
 <211> 19
 <212> DNA
 <213> artificial
 <220>
 <223> chemically synthesized
 <220>
 <221> misc_feature
 <222> (1)..(19)
 <223> primer
 <400> 4
 atgaggtgga cgtagtagt 19
 <210> 5
 <211> 26
 <212> DNA
 <213> artificial
 <220>
 <223> chemically synthesized
 <220>
 <221> misc_feature
 <222> (1)..(25)
 <223> primer
 <400> 5
 gaattcatgt gtgaaacaag cgcctt 26
 <210> 6
 <211> 24
 <212> DNA
 <213> artificial
 <220>
 <223> chemically synthesized
 <220>
 <221> misc_feature
 <222> (1)..(24)
 <223> primer
 <400> 6
 gtcgacagaa aaccagtgga tctg 24
 <210> 7
 <211> 18
 <212> DNA
 <213> artificial
 <220>

Tucap sequence listing.ST25.txt

<223> chemically synthesized

<220>
 <221> misc_feature
 <222> (1)..(18)
 <223> primer

<400> 7
 ccatggagaa ggctgggg

18

<210> 8
 <211> 20
 <212> DNA
 <213> artificial

<220>
 <223> chemically synthesized

<220>
 <221> misc_feature
 <222> (1)..(20)
 <223> primer

<400> 8
 caaagttgtc atggatgacc

20

<210> 9
 <211> 22
 <212> DNA
 <213> artificial

<220>
 <223> chemically synthesized

<220>
 <221> misc_feature
 <222> (1)..(22)
 <223> primer

<400> 9
 gaattcatgt gtgaaacaag cg

22

<210> 10
 <211> 24
 <212> DNA
 <213> artificial

<220>
 <223> chemically synthesized

<220>
 <221> misc_feature
 <222> (1)..(24)
 <223> primer

<400> 10
 gtcgatgtct atcttcacag cata

24

<210> 11
 <211> 265

Tucap sequence listing.ST25.txt

<212> PRT
 <213> Homo sapiens

<220>
 <221> PEPTIDE
 <222> (1)..(265)
 <223> TUCAP-1 N-terminal domain, amino acids 18-282 of TUCAP 1 protein

<400> 11

Met Cys Glu Thr Ser Ala Phe Tyr Val Pro Gly Val Ala Pro Ile Asn
 1 5 10 15

Phe His Gln Asn Asp Pro Val Glu Ile Lys Ala Val Lys Leu Thr Ser
 20 25 30

Ser Arg Thr Gln Leu Pro Tyr Glu Tyr Tyr Ser Leu Pro Phe Cys Gln
 35 40 45

Pro Ser Lys Ile Thr Tyr Lys Ala Glu Asn Leu Gly Glu Val Leu Arg
 50 55 60

Gly Asp Arg Ile Val Asn Thr Pro Phe Gln Val Leu Met Asn Ser Glu
 65 70 75 80

Lys Lys Cys Glu Val Leu Cys Ser Gln Ser Asn Lys Pro Val Thr Leu
 85 90 95

Thr Val Glu Gln Ser Arg Leu Val Ala Glu Arg Ile Thr Glu Asp Tyr
 100 105 110

Tyr Val His Leu Ile Ala Asp Asn Leu Pro Val Ala Thr Arg Leu Glu
 115 120 125

Leu Tyr Ser Asn Arg Asp Ser Asp Asp Lys Lys Lys Glu Lys Asp Val
 130 135 140

Gln Phe Glu His Gly Tyr Arg Leu Gly Phe Thr Asp Val Asn Lys Ile
 145 150 155 160

Tyr Leu His Asn His Leu Ser Phe Ile Leu Tyr Tyr His Arg Glu Asp
 165 170 175

Met Glu Glu Asp Gln Glu His Thr Tyr Arg Val Val Arg Phe Glu Val
 180 185 190

Ile Pro Gln Ser Ile Arg Leu Glu Asp Leu Lys Ala Asp Glu Lys Ser
 195 200 205

Ser Cys Thr Leu Pro Glu Gly Thr Asn Ser Ser Pro Gln Glu Ile Asp
 210 215 220

Pro Thr Lys Glu Asn Gln Leu Tyr Phe Thr Tyr Ser Val His Trp Glu
 8

225 Tucap sequence listing.ST25.txt 235 240

Glu Ser Asp Ile Lys Trp Ala Ser Arg Val Asp Thr Tyr Leu Thr Met
245 250 255

Ser Asp Val Gln Ile His Trp Phe Ser
260 265

<210> 12
<211> 15
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (1)..(15)
<223> amino acids 221-235 of TUCAP-1 protein

<400> 12

Ala Asp Glu Lys Ser Ser Cys Thr Leu Pro Glu Gly Thr Asn Ser
1 5 10 15

<210> 13
<211> 25
<212> RNA
<213> artificial

<220>
<223> chemically synthesized

<220>
<221> misc_feature
<222> (1)..(25)
<223> RNAi duplex

<400> 13
gagugacguc cagauccacu gguuu

25

<210> 14
<211> 25
<212> RNA
<213> Artificial

<220>
<223> Chemically synthesized

<220>
<221> misc_feature
<222> (1)..(25)
<223> RNA duplex

<400> 14
aaaccagugg aucuggacgu cacuc

25

<210> 15
<211> 50
<212> PRT
<213> Homo sapiens

Tucap sequence listing.ST25.txt

<220>

<221> PEPTIDE

<222> (1)..(50)

<223> amino acids 303-352 of TUCAP 1 protein

<400> 15

Arg Thr Leu Arg Lys Asp Ile Ala Asn Tyr Asn Lys Glu Asp Asp Ile
1 5 10 15

Glu Asp Thr Met Glu Glu Ser Gly Trp Lys Leu Val His Gly Asp Val
20 25 30

Phe Arg Pro Pro Gln Tyr Pro Met Ile Leu Ser Ser Leu Leu Gly Ser
35 40 45

Gly Ile
50