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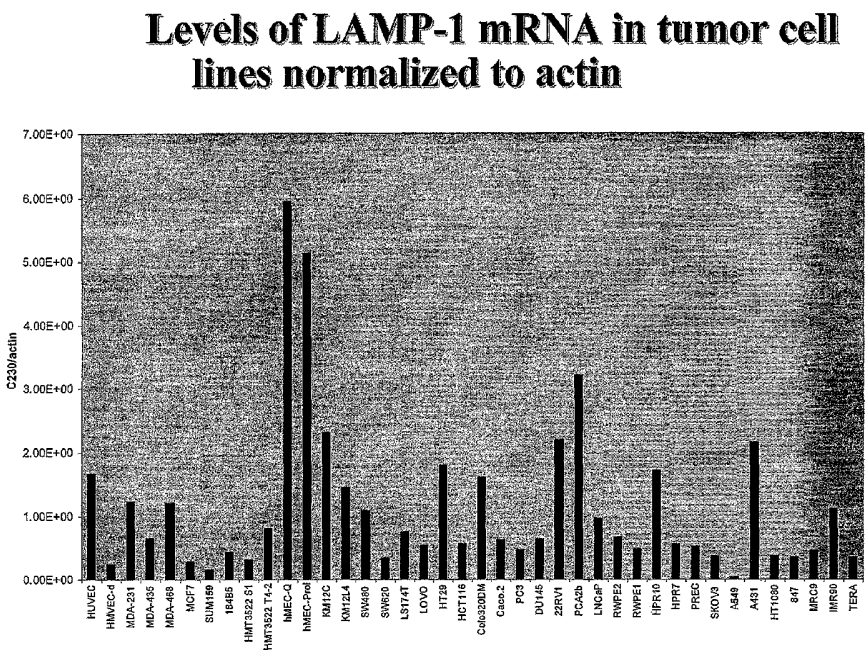
(54) Title: LAMP-1 MEMBRANE EXPRESSION BY CANCER CELLS



(57) Abstract: The invention provides methods of detecting a cancer cell and methods of reducing the proliferation of a cancer cell. For reducing the proliferation, the method consists of contacting the neoplastic cell with a cytotoxic or cytostatic binding agent specifically reactive with LAMP 1, a lysosome-associated membrane protein expressed on the cancer cell membrane. A method of reducing tumor growth through the targeting of a cytotoxic agent using an antibody specific for LAMP-1 is also provided, as is a method of detecting cancer cells expressing LAMP-1, to aid in diagnosis.

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LAMP-1 MEMBRANE EXPRESSION BY CANCER CELLS

Description

FIELD OF THE INVENTION

The invention relates generally to the field of cancer diagnostics and therapeutics, and specifically to the use of lysosomal-associated membrane protein 1 (LAMP-1) to detect cancer cells and to target therapeutic agents to cancer cells.

BACKGROUND OF THE INVENTION

Expression of lysosomal-associated membrane protein-1 (LAMP-1) has been generally described by Furuta, K. *et al.*, *Am. J. Pathol.* 159:449-455 (2001); Chen, J. W. *et al.*, *J. Biomed. Sci.* 8:365-374 (2001); Sarafian, V. *et al.*, *Int. J. Cancer* 75:105-111 (1998); Sawada, R. *et al.*, *J. Biol. Chem.* 268:12675-12681 (1993); Saitoh *et al.*, *J. Biol. Chem.* 267:5700-5711 (1992). However, as yet a clear picture of the expression of LAMP-1 in cancer cells has not emerged. Proteins preferentially displayed on the surface of cancer cells can help to distinguish cancer cells from normal cells of the same tissue, or normal cells of an organ to which the tumor has metastasized, thereby aiding in diagnosis and prognosis, as well as providing a target for therapeutic agents.

There exists a need in the art for improved diagnostic markers and methods of treating cancer, particularly colon, colorectal, and prostate cancer. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The invention provides a method of diagnosing cancer by contacting the cancer cell with an agent capable of specifically binding to LAMP-1.

In one embodiment of the method, the cancer cell is a human colon cancer cell taken from a colon tissue sample.

In another embodiment, the cancer cell is a human colon cancer cell taken from a non-colon tissue to which the colon cancer is suspected of metastasizing.

In specific embodiments of these methods, the agent capable of specifically binding to LAMP-1 is an antibody, more particularly a monoclonal antibody.

In one embodiment of the method using a monoclonal antibody, the monoclonal

antibody is generated by using cells of a patient's tumor biopsy as an immunogen in an animal, obtaining immune cells from the animal, and generating monoclonal antibodies. In a particular embodiment, the monoclonal antibodies are humanized.

In other embodiments, the antibodies are generated using a polypeptide comprising an antigenic region of LAMP-1. In a specific embodiment, the antigenic region comprises amino acids 323-340 of SEQ ID NO:1.

The invention further provides a method of reducing the proliferation of a neoplastic cell. The method consists of contacting the neoplastic cell with a cytotoxic or cytostatic binding agent specifically reactive with LAMP-1. The method further consists of administering to an individual containing a neoplastic cell population a cytotoxic or cytostatic binding agent specifically reactive with LAMP-1 that is expressed by the neoplastic cell population, wherein the cytotoxic or cytostatic binding agent is bound by the LAMP-1 and is optionally internalized into the intracellular compartment.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Figure 1 is a bar graph showing the levels of LAMP-1 mRNA in tumor cell lines normalized to actin.

Figure 2. Figure 2 is a bar graph showing the levels of LAMP-1 mRNA in tissues normalized to HPRT. The designations A and S after the names of the tissue types refers to two different sources of tissues.

Figure 3. Figure 3 shows the results of IHC analysis of LAMP-1 in normal colon and colon cancer tissue samples. In Fig. 3A, CC refers to colon cancer, and in Fig. 3B, CN refers to normal colon. In Fig. 3C, the data in the bar graph indicates that 30 out of 30 colon cancers showed staining, and 8 out of 8 normal colon tissues showed staining. In Fig. 3B, CC refers to colon cancer, and in Fig. 3C, CN refers to normal colon.

Figure 4. Figure 4 shows the results of IHC analysis of LAMP-1 in lung tissue. 6 out of 7 lung cancer samples showed staining, whereas zero out of 10 normal lung tissue samples showed staining.

Figure 5. Figure 5 (A-F) shows the results of IHC analysis of LAMP-1 in breast and prostate tissue. 5A: normal breast; 5B: breast cancer; 5C: prostate cancer; 5D: normal prostate; 5E: prostate cancer; 5F: prostate cancer.

Figure 6. Figure 6 is a series of images showing that LAMP-1 and membrane marker CD44 colocalized in a sample of human colon cancer tissue in one patient.

Figure 7. Figure 7 is a series of images showing that LAMP-1 and membrane marker

CD44 colocalized in a sample of human colon cancer tissue in a second patient.

Figure 8. Figure 8 is a series of images showing that LAMP-1 and membrane marker CD44 did not colocalize in an area of normal tissue within a frozen tissue section of human colon cancer tissue in one patient.

Figure 9. Figure 9 is a series of images showing that LAMP-1 and membrane marker CD44 did not colocalize in an area of normal tissue within a frozen tissue section of human colon cancer tissue in a second patient.

Figure 10. Figure 10 is an epitope map of the full length LAMP-1 protein, showing that the region of amino acids 323-340 exhibits an antigenicity value of greater than zero, while the remainder of the polypeptide exhibits an antigenicity value of less than zero.

Figure 11. Figure 11 is an amino acid sequence of full-length LAMP-1 protein, showing the signal sequence at amino acids 1-27, and the antigenic region at amino acids 323-340.

DETAILED DESCRIPTION OF THE INVENTION

The invention is directed towards improved methods of detecting cancer cells and of distinguishing cancer cells from normal cells of the same tissue type. The invention is also directed to methods for targeting immunoconjugates to neoplastic cell populations. The methods rely on the utilization of an antigen, LAMP-1, which is expressed on the neoplastic cells, to achieve high specificity for the target cell population.

Lysosomal-associated membrane protein 1 (LAMP-1) is a member of a family of glycoproteins that also includes LAMP-2. Both proteins are type I integral membrane proteins with a high oligosaccharide content. The proteins are heavily glycosylated, with about 50% of their mass being carbohydrate. LAMP-1 has a polypeptide core of approximately 40 kDa that consists of an intraluminal sequence with four cysteines, forming two disulfide bridges. Structurally, LAMP-1 has a transmembrane domain and a nine amino acid cytoplasmic sequence. LAMP-1 is localized primarily on the periphery of the lysosome, and is a major constituent of the lysosomal membrane and is speculated to be a "housekeeping" protein. LAMP-1 has been shown to bind galectin-3 and E-selectin (Sarafian, V. *et al.*, *Int. J. Cancer* 75:105-111, 1998), and may function as a ligand for selectin, mediating cell-cell adhesion. Depending on the extent of glycosylation, LAMP-1 can have a molecular weight of between 90 and 140 KDa.

LAMP-1 consists of 416 amino acid residues and 27 amino-terminal residues corresponding to a cleavable signal peptide. The major portion of LAMP-1 resides on the luminal side of the lysosome and is heavily glycosylated by N-glycans. LAMP-1 contains

19 potential N-glycosylation sites, which are clustered into two domains separated by a hinge-like structure enriched with proline and serine. The two domains of LAMP-1 on each side of the hinge region are homologous to each other. LAMP-1 has one transmembrane domain consisting of 24 hydrophobic amino acids near the COOH terminus, and contains a short cytoplasmic segment composed of 11 amino acid residues at the COOH-terminal end.

The LAMP-1 polypeptide sequence, as shown in SEQ ID NO:3, comprises an antigenic region at amino acid positions 323-340. This region is encoded by nucleotides 1157-1210 of SEQ ID NO:2. The antigenic region does not contain predicted N- or O- glycosylation sites. The antigenic region was identified by performing an antigenicity analysis of full-length LAMP-1 (Figure 10).

Transformed (cancerous) cells exhibit increased sialylation and β1-6-linked branching of complex-type asparagine (Asn)-linked oligosaccharides. Increased amounts of N-glycans are often associated with an increased amount of poly-N-acetyllactosamine. LAMP-1 proteins are known to be major carriers for poly-N-acetyllactosamines. The content of poly-N-acetyllactosamines in LAMP-1 have been correlated with tumor differentiation and metastatic potential (Siatoh *et al.*, *J. Biol. Chem.* 267:5700-5711, 1992; Sawada, R. *et al.*, *J. Biol. Chem.* 268:12675-12681, 1993). High levels of LAMP-1 have been found on the cell surface of highly metastatic tumor cells, and higher levels of LAMP-1 have been found on surfaces of highly metastatic colon carcinoma cells as compared to low metastatic cell lines. However, the present invention shows, for the first time, that LAMP-1 cell surface expression is specific enough to tumor cells, in contrast to normal cells of the same tissue type, to allow its use for diagnosis and for specifically targeting therapeutic agents to the tumor cells.

According to the invention, LAMP-1 is consistently expressed on the surface of colon cancer cells, but not on the surface of normal colon cells. This distinction has been shown for the first time by the use of immunofluorescence staining and confocal microscopy analysis. Using frozen tissue sections of normal colon tissue and colon cancer tissue, LAMP-1 expressed on the cell surface was detected and labeled using a monoclonal antibody specific for LAMP-1, as described in the Examples. A separate monoclonal antibody was used as a membrane marker to detect CD44, a membrane protein. Expression of LAMP-1 on the cell surface was indicated by the colocalization of LAMP-1 and CD44 in the colon tumor samples, but not in the samples from normal colon tissue. This method therefore is the first indication that LAMP-1 is specifically expressed on the cell surface of colon cancer cells, thus providing both a marker and an improved method for targeting therapeutic agents specifically to the colon cancer cells.

Because of the varying extent of glycosylation of LAMP-1, it is preferable to raise

antibodies to the LAMP-1 expressed on the surface of a patient's tumor cells, in order to better target the antibodies to the tumor cells in the body, and also to optimally distinguish between tumor cells and normal cells on the basis of LAMP-1 expression on the cell surface. In one exemplary method, whole human colon cancer cells expressing LAMP-1 on the cell surface are injected into Lewis rats, following the immunization protocol of three injections two weeks apart, with about 10^6 cells per injection. The route of immunization is intrasplenic. The spleen cells of the rat are removed and lymphocytes are fused with mouse SP/20 myeloma cell line. Resulting clones are assayed for the production of antibodies specifically recognizing the LAMP-1 expressed by the tumor cells that were used as the source for the whole cell immunogen. Antibodies that specifically bind are preferable for the diagnostic and targeting functions as described herein, for that particular patient.

The identification of an antigenic region of LAMP-1 herein will aid in the production of compositions useful as vaccines and as binding agents for targeting therapeutic agents to LAMP-1-expressing cells, such as cancer cells. The invention will also aid in the production of improved DNA vaccines. DNA vaccine potency can be enhanced by co-administering DNA encoding LAMP-1. (Kim, T.W. *et al.*, *J. Immunol.* 15:2970-2976, 2003.) With the present disclosure of the LAMP-1 antigenic region (amino acids 323-340), DNA encoding an antigenic portion of LAMP-1, instead of the entire protein, can be generated. Such DNA comprises a nucleotide sequence corresponding to nucleotides at positions 1157-1210 of SEQ ID NO:2. One or more of these bases can be substituted, as long as the encoded amino acid sequence is capable of specifically binding to an antibody that recognizes SEQ ID NO:3, which corresponds to the LAMP-1 antigenic region as described herein.

Alternatively, one can also raise anti-LAMP-1 antibodies that bind to polypeptide epitope that is expressed on the cell surface. A preferred epitope comprises amino acid sequence SLRALQATVGNSYKCNAE (SEQ ID NO:3).

Analysis of colon tissue, as described in the Examples, showed a consistent pattern of LAMP-1 expression on the surface of colon cancer cells, but not on the surface of normal colon cells. LAMP-1 therefore is an appropriate target for diagnostic and targeting use in colon cancer. Described herein are methods for targeting agents to cancer cells expressing LAMP-1, and methods of detecting cancer cells by analyzing the cell surface expression of LAMP-1. Prior to discussion of specific methods, terms used in this application are described below.

The terms "cancer", "neoplasm", "tumor", and "carcinoma", are used interchangeably herein to refer to cells that exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. In general,

cells of interest for detection or treatment in the present application include precancerous (*e.g.*, benign), malignant, metastatic, and non-metastatic cells.

As used herein, the term "binding agent" refers to a molecule that exhibits specific binding activity towards LAMP-1. Such a binding molecule can include a variety of different types of molecules including, for example, macromolecules and small organic molecules. The type of binding agent selected will depend on the need. Small molecule binding agents can include, for example, receptor ligands, antagonists and agonists. Macromolecules can include, for example, peptide, polypeptide and protein, nucleic acids encoding polypeptide binding agents, lectins, carbohydrate and lipids. It is understood that the term includes fragments and domains of the agent so long as binding function is retained. Similarly, the boundaries of the domains are not critical so long as binding activity is maintained. In the specific example where the binding agent is a peptide, polypeptide or protein, such binding proteins can include monomeric or multimeric species. Heteromeric binding proteins are a specific example of multimeric binding proteins. It is understood that when referring to multimeric binding proteins that the term includes fragments of the subunits so long as assembly of the polypeptides and binding function of the assembled complex is retained. Heteromeric binding proteins include, for example, antibodies and fragments thereof such as Fab and $F(ab')_2$ portions.

Another example of binding proteins is an antibody. "Antibodies" and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to an antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. The term "antibody" is used in the broadest sense and covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, single chain and antibody fragments (including F(ab), $F(ab)_2$, and Fv) so long as they exhibit the desired biological activity, in this case, binding LAMP-1.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations that are typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the homogeneous culture, uncontaminated by other immunoglobulins with

different specificities and characteristics.

The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., Nature, 352:624-628 (1991) and Marks et al., J. Mol. Biol., 222:581-597 (1991), for example.

The terms "cytotoxic" and "cytostatic" when used to characterize a binding agent are intended to mean that the binding agent exhibits or has been modified to exhibit cytotoxic or cytostatic properties. For example, there are many known toxins or drugs that are known by those skilled in the art to exhibit these properties. Specific examples of cytotoxic and cytostatic agents include, for example, pokeweed antiviral protein, abrin, ricin and each of their A chains, doxorubicin, cisplastin, Iodine-131, Yttrium-90, Rhenium-188, Bismuth-212, Taxol, 5-Fluorouracil VP-16, Bleomycin, methotrexate, vindesine, adriamycin, vincristine, vinblastine, BCNU, mitomycin and cyclophosphamide and certain cytokines such as TNF and TNF-β. Thus, cytotoxic or cytostatic agents can include, for example, radionuclides, chemotherapeutic drugs, proteins and lectins. Any of these agents can be attached to a binding agent for the cytoplasmic targeting of therapeutic agents to neoplastic cells.

As used herein, the term "specifically reactive" when used in reference to a binding agent refers to the discriminatory binding of the binding agent to LAMP-1. For such binding to be discriminating, the binding agent will not substantially cross-react, or can be made not to substantially cross-react, with other surface markers which are not LAMP-1. Specific reactivity can include binding properties such as binding specificity, binding affinity and binding avidity.

For example, specifically reactive anti-LAMP-1 antibodies will LAMP-1 with greater affinity than other molecules. Preferably, the relative binding affinity of LAMP-1 over a related protein will be at least 5/1, more preferably at least 10/1, 20/1, 50/1, 100/1 or 1000/1, 10,000/1 or the antibody will not detectably bind to proteins other than the target.

The invention provides a method of reducing the proliferation of a neoplastic cell. The method consists of contacting the neoplastic cell with a cytotoxic or cytostatic binding agent which is specifically reactive with LAMP-1.

Among the many phenotypes associated with neoplasia, a large percentage result from the deregulation of the cell cycle that leads to enhanced proliferative phenotype. Apart from the

altered expression or activity of the regulatory proteins involved in such cell cycle control, there are relatively few pronounced molecular changes that are known. Although some differences, such as altered glycosylation, have been observed on neoplastic cells, in general there are very few molecular markers that are specifically expressed on the surface of the neoplastic cell and therefore available for immunotherapeutic targeting. In contrast, many antigens that have been characterized in regard to tumor specific expression have been found to be only modestly selective between neoplastic and normal phenotypes. For these reasons, it can be extremely laborious to find antigens that are specific to neoplastic cells.

The methods of the invention target cytotoxic or cytostatic agents to neoplastic cells through the use of binding agents that are specific to LAMP-1. As defined above, such binding agents can be essentially any molecule, including peptide, polypeptide and protein or other macromolecules or binding compounds which exhibit specific binding activity toward LAMP-1.

Cytotoxic or cytostatic agents are attached to the binding agents by a variety of methods known in the art. Attachment, coupling or conjugation can be accomplished by, for example, covalent bond formation; however, other means known in the art can be equally applied as well. Essentially any type of coupling methodology will work so long as conditions are used to maintain the functions of both of the binding agent and the cytotoxic or cytostatic agent. Such methods are well known in the art and are described in, for example, Harlow et al. (Antibodies: A Laboratory Manual, Cold Spring Harbor (1988)). See also, WO 00/52031, US 5,635,483, and US 6,214,345 B1.

For example, the covalent bond can be formed by way of carbodiimide, glutaraldehyde, heterobifunctional cross-linkers, and homobifunctional cross-linkers. The cross-linking of proteins can additionally be accomplished by using reactive groups within the individual protein such as carbohydrate, disulfide, carboxyl or amino groups. Coupling can be accomplished by oxidation or reduction of the native protein, or treatment with an enzyme, for example.

Similarly, numerous different cytotoxic and cytostatic agents are known by those skilled in the art. Selection of which cytotoxic or cytostatic agent to use will depend on the need and will be known, or can be determined by those skilled in the art.

The cytotoxic or cytostatic agents can range from small organic molecules to large biologically active proteins and other macromolecules. To reduce proliferation of a neoplastic cell, the cytotoxic or cytostatic agent and the binding agent are attached, or conjugated, to one another to produce a cytotoxic or cytostatic binding agent. The binding agent is chosen so as to be specifically reactive with LAMP-1 present on the surface of the neoplastic cell. These therapeutic binding agents are then placed in contact with the neoplastic cells and allowed to

bind LAMP-1.

Another approach is using antibodies that activate human effector cells to kill the LAMP-1 expressing tumor cells. "Human effector cells" are leukocytes that express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and carry out antigen-dependent cell-mediated cyotoxicity (ADCC) effector function. Examples of human leukocytes that mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred.

The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody.

The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and Fcγ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see Daeron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)).

"Complement dependent cytotoxicity" or "CDC" refer to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (Clq) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed.

The methods described herein for reducing the proliferation of a neoplastic cell are applicable for *in vitro* diagnosis of neoplastic cells or the testing of various agents applicable to both the *ex vivo* and *in vivo* targeting of therapeutic agents to neoplastic cell populations. Such methods are useful, for example, for testing the cytotoxic or cytostatic effects of specific agents *in vitro*, bone marrow purging *ex vivo* and for inhibiting the growth of single or small

populations of metastatic cells at secondary tumor sites. Similarly, the methods described above are equally applicable for inhibiting proliferation and/or viability of larger neoplastic cell populations or solid tumors, for example. In these specific examples, the cytotoxic or cytostatic binding agent is administered in a therapeutically effective dose so as to circulate and bind LAMP-1 expressed on the cell surface of the tumor cell.

The cytotoxic or cytostatic agents are administered to an individual exhibiting or at risk of exhibiting cells having a neoplastic phenotype. Definite clinical diagnosis of neoplasia warrants the administration of one or more cytotoxic or cytostatic binding agents to the relevant neoplastic cell specific antigen. Prophylactic applications are warranted in cases where there is a genetic disposition to develop neoplasia or where there is a possibility that secondary metastasis or recurrence of the original growth can occur.

Cytotoxic or cytostatic binding agents can be administered in many possible formulations, including pharmaceutically acceptable media. In the case of a short peptide, the peptide can be conjugated to a carrier, for example, in order to increase its stability within the circulatory system. Antibodies are advantageous for use as a binding agent since they are naturally long-lived proteins of the circulatory system. Antibodies can be produced in a variety of mammals and then genetically engineered to resemble proteins of human origin and in this way avoid endogenous host defense mechanisms. Methods for humanization of antibodies are well known in the art and are described, for example, in Winter and Harris, *Immunol. Today*, 14:243-246 (1993); Winter and Harris, *TIPS*, 14:139-143 (1993) and Couto et al. *Cancer Res.*, 55:1717-1722 (1995).

In specific embodiments of the present invention, chimeric, humanized, and human anti-LAMP-1 monoclonal antibodies are provided. "Chimeric" antibodies are antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)).

The phrase "humanized antibody" refers to an antibody derived from a non-human antibody, typically a mouse monoclonal antibody. Alternatively, a humanized antibody may be derived from a chimeric antibody that retains or substantially retains the antigen binding properties of the parental, non-human, antibody but which exhibits diminished immunogenicity

as compared to the parental antibody when administered to humans. The phrase "chimeric antibody," as used herein, refers to an antibody containing sequence derived from two different antibodies (*see, e.g.*, U.S. Patent No. 4,816,567) that typically originate from different species. Most typically, chimeric antibodies comprise human and murine antibody fragments, generally human constant and mouse variable regions. The antibodies are prepared by injecting a mammal with tumor cells expressing LAMP-1 on the cell surface, collecting the immune cells from the mammal, and preparing monoclonal antibodies as is known in the art.

Because humanized antibodies are less immunogenic in humans than the parental mouse monoclonal antibodies, they can be used for the treatment of humans with far less risk of anaphylaxis. Thus, these antibodies may be preferred in diagnostic and therapeutic applications that involve *in vivo* administration to a human.

Humanized antibodies may be achieved by a variety of methods including, for example: (1) grafting the non-human complementarity determining regions (CDRs) onto a human framework and constant region (a process referred to in the art as "humanizing"), or, alternatively, (2) transplanting the entire non-human variable domains, but "cloaking" them with a human-like surface by replacement of surface residues (a process referred to in the art as "veneering"). In the present invention, humanized antibodies will include both "humanized" and "veneered" antibodies. These methods are disclosed in, *e.g.*, Jones *et al.*, *Nature 321:522-525* (1986); Morrison *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, 81:6851-6855 (1984); Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1988); Verhoeyer *et al.*, *Science* 239:1534-1536 (1988); Padlan, *Molec. Immun.* 28:489-498 (1991); Padlan, *Molec. Immunol.* 31(3):169-217 (1994); and Kettleborough, C.A. *et al.*, *Protein Eng.* 4(7):773-83 (1991) each of which is incorporated herein by reference.

The phrase "complementarity determining region" refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. *See, e.g.*, Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987); Kabat *et al.*, U.S. Dept. of Health and Human Services NIH Publication No. 91-3242 (1991). The phrase "constant region" refers to the portion of the antibody molecule that confers effector functions. In the present invention, mouse constant regions can be substituted by human constant regions. The constant regions of the subject humanized antibodies are derived from human immunoglobulins. The heavy chain constant region can be selected from any of the five isotypes: alpha, delta, epsilon, gamma or mu.

One method of humanizing antibodies comprises aligning the non-human heavy and light chain sequences to human heavy and light chain sequences, selecting and replacing the

non-human framework with a human framework based on such alignment, molecular modeling to predict the conformation of the humanized sequence and comparing to the conformation of the parent antibody. This process is followed by repeated back mutation of residues in the CDR region that disturb the structure of the CDRs until the predicted conformation of the humanized sequence model closely approximates the conformation of the non-human CDRsframeworks of the parent non-human antibody. Such humanized antibodies may be further derivatized to facilitate uptake and clearance, *e.g.*, via Ashwell receptors. *See, e.g.*, U.S. Patent Nos. 5,530,101 and 5,585,089 which patents are incorporated herein by reference.

Humanized antibodies to LAMP-1 can also be produced using transgenic animals that are engineered to contain human immunoglobulin loci. For example, WO 98/24893 discloses transgenic animals having a human Ig locus wherein the animals do not produce functional endogenous immunoglobulins due to the inactivation of endogenous heavy and light chain loci. WO 96/30498 discloses the use of the Cre/Lox system to modify the immunoglobulin locus in a mammal, such as to replace all or a portion of the constant or variable region to form a modified antibody molecule. WO 94/02602 discloses non-human mammalian hosts having inactivated endogenous Ig loci and functional human Ig loci. U.S. Patent No. 5,939,598 discloses methods of making transgenic mice in which the mice lack endogenous heavy chains, and express an exogenous immunoglobulin locus comprising one or more xenogeneic constant regions.

Using a transgenic animal described above, an immune response can be produced to a selected antigenic molecule, preferably expressed on the surface of a tumor cell, such as a colon cancer cell, and antibody producing cells can be removed from the animal and used to produce hybridomas that secrete human monoclonal antibodies. Immunization protocols, adjuvants, and the like are known in the art, and are used in immunization of, for example, a transgenic mouse as described in WO 96/33735. This publication discloses monoclonal antibodies against a variety of antigenic molecules including IL-6, IL-8, TNFaTNFα, human CD4, L-selectin, gp39, and tetanus toxin. The monoclonal antibodies can be tested for the ability to inhibit or neutralize the biological activity or physiological effect of the corresponding protein. WO 96/3373 discloses that monoclonal antibodies against IL-8, derived from immune cells of transgenic mice immunized with IL-8, blocked IL-8 induced functions of neutrophils. Human monoclonal antibodies with specificity for the antigen used to immunize transgenic animals are also disclosed in WO 96/34096.

Another method of generating and screening human antibodies or antibody fragments is from antibody phage libraries generated using the techniques described in McCafferty *et al., Nature,* 348:552-554 (1990). Clackson *et al., Nature,* 352:624-628 (1991) and Marks *et al., J.*

Mol. Biol., 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al., BiolTechnology,* 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al., Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The LAMP-1 antibodies of the present invention are said to be immunospecific or specifically binding if they bind to LAMP-1 with a K_a of greater than or equal to about 10^4M^{-1}, preferably of greater than or equal to about 10^5M^{-1}, more preferably of greater than or equal to about 10^6M^{-1} and still more preferably of greater than or equal to about 10^7M^{-1} and still more preferably of greater than or equal to 10^9M^{-1}. Such affinities may be readily determined using conventional techniques, such as by equilibrium dialysis; by using the BIAcore 2000 instrument, using general procedures outlined by the manufacturer; by radioimmunoassay using ^{125}I-labeled LAMP-1; or by another method known to the skilled artisan. The affinity data may be analyzed, for example, by the method of Scatchard *et al., Ann N.Y. Acad. Sci.*, 51:660 (1949). Thus, it will be apparent that preferred anti-LAMP-1 will exhibit a high degree of specificity for LAMP-1 and will bind with substantially lower affinity to other molecules.

The anti-LAMP-1 antibodies of the present invention can bind to amino-acid- residues-specific-epitopes, carbohydrate-specific epitopes, or epitopes formed by both amino acid residues and carbohydrate portions of the molecule, as expressed by the LAMP-1 bearing tumor cells. If the anti-LAMP antibody is carries a cytotoxic or cytostatic agent, it might be preferable that the antibody binds to an epitope that internalizes the antibody-LAMP-1 receptor complex. If the anti-LAMP-1 antibody is to work through ADCC and CDC, then it is preferable that the anti-LAMP-1 antibody remains on the surface of the target tumor cell until the antibody's Fc region binds to effector cells. Methods for determining whether an antibody bound to a cognate cell surface antigen remains on a cell surface or is internalized are well known in the art.

By the present methods, compositions comprising antibodies to LAMP-1 may be administered parenterally, topically, orally, or locally for therapeutic treatment. Preferably, the compositions are administered orally or parenterally, i.e., intravenously, intraperitoneally, intradermally or intramuscularly. Thus, this invention provides methods that employ compositions for administration that comprise one or more antibodies to LAMP-1 in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.4% saline, 0.3% glycine and the like, and may

include other proteins for enhanced stability, such as albumin, lipoprotein, globulin, etc., subjected to mild chemical modifications or the like.

LAMP-1 antibodies of the invention will often be prepared substantially free of other naturally occurring immunoglobulins or other biological molecules. Preferred LAMP-1 antibodies will also exhibit minimal toxicity when administered to a mammal afflicted with, or predisposed to suffer from cancer and/or cancer metastasis, specifically colon cancer.

The compositions of the invention may be sterilized by conventional, well-known sterilization techniques. The resulting solutions may be packaged for use or filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride and stabilizers (*e.g.*, 1-20% maltose, etc.).

The LAMP-1 antibodies of the present invention may also be administered via liposomes. Liposomes, which include emulsions, foams, micelles, insoluble monolayers, phospholipid dispersions, lamellar layers and the like, can serve as vehicles to target the LAMP-1 antibodies to a particular tissue as well as to increase the half life of the composition. A variety of methods are available for preparing liposomes, as described in, *e.g.*, U.S. Patent Nos. 4,837,028 and 5,019,369, which patents are incorporated herein by reference.

Compositions of the invention are administered to a mammal already suffering from, or predisposed to, cancer and/or cancer metastasis in an amount sufficient to prevent or at least partially arrest the development of cancer metastasis. An amount adequate to accomplish this is defined as a "therapeutically effective dose." More specifically, a "therapeutically effective dose", in reference to the treatment of tumor, refers to an amount capable of invoking one or more of the following effects: (1) inhibition, to some extent, of tumor growth, including, slowing down and complete growth arrest; (2) reduction in the number of tumor cells; (3) reduction in tumor size; (4) inhibition (i.e., reduction, slowing down or complete stopping) of tumor cell infiltration into peripheral organs; (5) inhibition (i.e., reduction, slowing down or complete stopping) of metastasis; (6) enhancement of anti-tumor immune response, which may, but does not have to, result in the regression or rejection of the tumor; and/or (7) relief, to some extent, of one or more symptoms associated with the disorder.

Effective amounts of an agent targeted to a tumor cell by means of a LAMP-1 antibody will vary and depend on the severity of the disease and the weight and general state of the

patient being treated, but generally range from about 1.0 μg/kg to about 100 mg/kg body weight, with dosages of from about 10 μg/kg to about 10 mg/kg per application being more commonly used. Administration is daily, weekly or less frequently, as necessary depending on the response to the disease and the patient's tolerance of the therapy. Maintenance dosages over a prolonged period of time may be needed, and dosages may be adjusted as necessary.

Single or multiple administrations of the compositions can be carried out with the dose levels and pattern being selected by the treating physician. In any event, the formulations should provide a quantity of an agent targeted to LAMP-1 sufficient to effectively prevent or minimize the severity of cancer and/or cancer metastasis. The compositions of the present invention may be administered alone or as an adjunct therapy in conjunction with other therapeutics known in the art for the treatment of cancer and/or cancer metastasis.

The cytotoxic or cytostatic binding agents are administered by conventional methods, in dosages that are sufficient to effect binding of the neoplastic cell-specific internalizing antigen. Such dosages are known or can be easily determined by those skilled in the art. Administration can be accomplished by, for example, intravenous, interperitoneal or subcutaneous injection. Administration can be performed in a variety of different regimens that include single high dose administration or repeated small dose administration or a combination of both. The dosing will depend on the type of neoplasia, progression of the disease and overall health of the individual and will be known or can be determined by those skilled in the art.

The cytotoxic or cytostatic binding agent can be administered to an individual either singly or in a cocktail containing two or more cytotoxic or cytostatic binding agents, other therapeutic agents, compositions, or the like, including, for example, immunosuppressive agents, potentiators and side-effect relieving agents. Immunosuppressive agents include for example, prednisone, DECADRON (Merck, Sharp & Dohme, West Point, Pa.), cyclophosphamide, cyclosporine, 6-mercaptopurine, methotrexate, azathioprine and i.v. gamma globulin or their combination. Potentiators include, for example, monensin, ammonium chloride and chloroquine. All of these agents are administered in generally accepted efficacious dose ranges such as those disclosed in the Physician's Desk Reference.

The cytotoxic or cytostatic binding agents can be formulated into, for example, injectable or topical preparations for administration. Parenteral formulations are known and are suitable for use in the invention, preferably for i.m. or i.v. administration. Formulations containing therapeutically effective amounts of the cytotoxic or cytostatic binding agents can be sterile liquid solutions, liquid suspensions or lyophilized versions and can additionally contain stabilizers or excipients for example. Therapeutically effective doses of the cytotoxic or

cytostatic binding agents can be, for example, in a range of from about less then 0.01 mg/kg to about greater than 10 mg/kg body weight of the treated individual administered over several days to two weeks by daily intravenous infusion.

The cytotoxic or cytostatic binding agents can be formulated into topical preparations for local therapy by including them in a dermatological vehicle. The amount of agent to be administered will depend upon the vehicle selected, the clinical condition of the patient, the systemic toxicity and the stability of the anti-T cell immunotoxin in the formulation. Suitable vehicles include for example, gels or water-in-oil emulsions using mineral oils, petrolatum and the like.

Cytotoxic or cytostatic binding agents can also be administered by aerosol to achieve localized delivery to the lungs. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the agents or derivatives thereof. Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the cytotoxic or cytostatic binding agents together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers will vary depending upon the requirements for the particular binding agent but include, for example, nonionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. The formulations also can include mucolytic agents as well as bronchodilating agents. The formulations will be sterile. Aerosols generally will be prepared from isotonic solutions. The particles optionally include normal lung surfactants.

Also provided is a kit comprising an anti-LAMP-1 antibody, or an agent capable of specifically binding to LAMP-1, and instructions for delivering the anti-LAMP-1 antibody or agent to an individual suffering from cancer, including colon cancer, metastatic colon cancer, or lung cancer. The anti-LAMP-1 antibody or the agent may be coupled to a cytotoxic or cytostatic agent. The invention also provides for use of an anti-LAMP-1 antibody, or an agent capable of specifically binding to LAMP-1, in the preparation of a medicament for treating cancer, including colon cancer, metastatic colon cancer, or lung cancer in a mammal, particularly a human subject, wherein the agent or the antibody may be coupled with a cytotoxic or cytostatic agent.

LAMP-1, as generally defined herein, may also be used as a cancer vaccine according to methods known in the art. Cancer vaccines are described in U.S. Patent Publication 2002 0006900 A1 (January 17, 2002; U.S. Patent No. 5,993,829 (November 30, 1999) and U.S. Patent No. 6,146,632 (November 14, 2000). Further examples of cancer vaccines include Gilewski *et*

al., *Clin. Cancer Res.* 6:1693-1701 (2000); Berinstein, N. L., *Jour. Clin. Oncol.* 20:2197-2207 (2002); and Ragupathi, G. *et al.*, *P.N.A.S.* 99:13699-13704 (2002), which discusses methods for preparing and using carbohydrate-based cancer vaccines. These patents and publications are incorporated by reference.

It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also included within the definition of the invention provided herein. Accordingly, the following examples are intended to illustrate but not limit the present invention.

EXAMPLE 1

LAMP-1 Expression in Normal and Cancer Tissue by IHC

Figure 1 shows that LAMP-1 mRNA levels in tumor cells, normalized to actin, show a wide variation. Figure 2 shows the LAMP-1 levels in tissues normalized to HPRT. Thus, it was of importance to determine if such variation was also observed in the cell surface of tumor cells.

First, IHC analysis of LAMP-1 was performed on colon cancer samples and normal colon samples. As shown in Figure 3, 30 out of 30 colon cancer samples showed staining, and 8 out of 8 samples of normal colon tissue showed staining. Thus, IHC analysis is not adequate for differentiating between normal and cancerous colon tissue.

Immunohistochemistry ("IHC") analysis can be performed on samples consisting of paraffin sections, uncooked paraffin tissues, and frozen tissues. This analysis can be performed in the following manner.

Paraffin sections are de-paraffinized in Xylene and then washed through a series of graded alcohol washes (100% Reagent alcohol, followed by 95%, 85%, 75% Reagent alcohol). The samples are then re-hydrated in 1x PBS for five minutes followed by a distilled water wash (2-3 quick rinses).

Antigen retrieval is accomplished in a 1X *BioGenex* Citra Plus solution brought to a boil and then simmered for 13 minutes, followed by a cooling at room temperature for 30 minutes. The sample is then washed in distilled water three times.

To block peroxide, a 3% peroxide solution is applied for 10 minutes at room temperature, followed with two rinses in distilled water and then a final wash in 1X PBS (2 x 2 minutes). Avidin is blocked with a 10 minute room temperature incubation in *Zymed* Avidin, followed by a 1X PBS wash (2 x 3 minutes). Biotin is blocked in *Zymed* Biotin at room temperature for 10 minutes, followed by a 1X PBS wash (2 x 3 minutes). Protein is blocked in a

1X solution of *BioGenex* Power Block diluted to 1X in distilled water (8 minute incubation at room temperature), followed by a 1X PBS wash.

The primary antibody is applied for 1 hour at room temperature followed by 3 1X PBS washes (3-5 minutes each). The secondary antibody is applied for 10-11 minutes at room temperature followed by a PBS wash (3 x 3 minutes).

The tissues are labeled with a horseradish peroxidase (HRP) label with a 10-11 minute incubation followed by a 1X PBS wash (3 x 3 minutes). The substrate is a *BioGenex* hydrogen peroxide substrate applied in a 10 minute room temperature incubation followed by several washes of distilled water. The counterstain is applied with a 1 minute room temperature incubation of hematoxylin. The counterstain is followed by two distilled water washes and the samples are then incubated in 1X PBS for 1 minute. The PBS is removed with several distilled water rinses. Sections are mounted using *BioGenex* SuperMount and air dried overnight at room temperature.

For samples of uncooked paraffin tissues, the slides are first cooked at 55-60°C for 1 hour and then cooled. The above steps can then followed in the same manner. For samples of frozen tissues, the de-paraffination and antigen retrieval steps are not necessary, and the IHC process begins at the peroxide block step.

IHC analysis was also performed on lung cancer and normal lung tissues. 6 out of 7 lung cancers showed staining for LAMP-1, whereas zero out of 10 normal lung samples showed staining. (Figure 4.4). IHC analysis of LAMP-1 was also performed on normal breast samples, breast cancer samples, normal prostate samples, and prostate cancer samples, and the results are shown in Figure 5.

EXAMPLE 2

Immunofluorescence Staining for LAMP-1

Because the previously used methods were not efficient at using LAMP-1 detection to distinguish between normal and cancer tissue in the colon, a combination of immunfluorescence staining and confocal analysis was performed. Frozen tissue sections of colon cancer were used. The LAMP-1 was targeted using the monoclonal antibody CD107aH4A3, which is specific for LAMP-1. The monoclonal antibody CD44 (RDI-CD154PE) was used as a membrane marker. CD44 was directly conjugated to Alexa Fluor-568, which fluoresces as red. Both monoclonal antibodies were obtained from RDI (Research Diagnostics, Inc., Pleasant Hill Road, Flanders, NJ 07936). Alexa Fluor anti-IgG-488 (green) was used as the secondary antibody, and DAPI

was used as the nuclei stain. Confocal analysis was performed using a Zeiss LSM510 confocal microscope, and the confocal images were analyzed using Carl Zeiss AxioVision software.

Immunofluoresence was performed as follows. The cells were blocked in a solution of PBS containing 5% goat serum (GS) and 1% BSA for one hour. The primary antibody was applied diluted in PBS with 5% GS and 0.1% BSA and incubated for one hour at room temperature. The cells were washed twice for 15 minutes each time in PBS + 0.05% Tween. The secondary antibody was applied diluted in PBS, 5% GS and 0.1% BSA, and incubated for 15 – 20 minutes at room temperature in the dark. The cells were washed three times for 15 minutes each in PBS + 0.05% Tween. Washing for up to an hour could be performed to eliminate background. A final rinse in PBS was performed, and the stained cells were mounted with Vectashield and diluted DAPI.

As shown in Figures 6 and 7, in two different exemplary human patient samples, LAMP-1 colocalized with the membrane marker CH44 in human colon cancer. In contrast, as shown in Figures 8 and 9, areas of normal tissue within sections of colon cancer were characterized by a distinct lack of colocalization of LAMP-1 with CD44.

LAMP-1 is a major component of lysosomes, and is upregulated in tumors compared to normal tissues. The confocal analysis performed according to this example demonstrates for the first time that LAMP-1 is expressed on the surface of cancer cells but not on the surface of normal cells, as detected in colon cancer and normal colon tissue.

EXAMPLE 3

Preparation and Use of Antibodies for Specifically Identifying Cancer Cells Expressing LAMP-1

Because LAMP-1 has been identified herein as a marker for colon cancer by immunofluorescence using confocal analysis, antibodies to LAMP-1 expressed in cancer cells of a patient can be used for a variety of diagnostic and therapeutic applications.

This Example describes methods of raising antibodies and preparing monoclonal antibodies to LAMP-1 using whole tumor cells. Further, the method includes the delivery of cytotoxic or cytostatic agents attached to antibodies specific to LAMP-1 found on the surfaces of neoplastic cells. Additionally, the method includes detecting metastases of tumor cells expressing LAMP-1 by antibody bound-LAMP-1. The antibodies can be used for determining the presence or purification of the LAMP-1 glycoproteins of the present invention. With respect to the detection of such glycoproteins, the antibodies can be used for in vitro diagnostic or in vivo imaging methods. There are a number of antibodies which recognize cell surface antigens,

such as LAMP-1, reported to be preferentially expressed on neoplastic cells. These antibodies are increasingly being applied in the clinic as diagnostic tools and as potential therapeutic treatments.

First, antibodies are raised to LAMP-1. Antibodies having specific reactivity with the LAMP-1 glycoproteins of the invention can be produced by any method known in the art. For example, polyclonal and monoclonal antibodies can be produced by methods well-known in the art, as described, for example, in Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, 1988), which is incorporated herein by reference. Antibodies can be generated by inoculation of an animal. The LAMP-1 of the present invention can be used as the immunogen in generating antibodies. For example, antibodies against human proteins are frequently raised in rabbits. The sera of such immunized animals contain a mixture of antibodies (produced by different lymphocytes) that react against multiple sites on the immunizing antigen. However, monoclonal antibodies can also be produced by culturing clonal lines of B lymphocytes from immunized animals such as mice. Because each lymphocyte is programmed to produce only a single antibody, a clonal line of lymphocytes produces a monoclonal antibody that recognizes only a single antigenic determinant, such as LAMP-1, thereby providing a highly specific immunological reagent.

Although antibodies can be raised against proteins purified from cells, other materials may also be used for immunization. Antibodies can be produced from intact cells expressing LAMP-1, by immunizing animals with intact cells to raise antibodies against proteins, such as LAMP-1, expressed by a specific cell type, e.g., a cancer cell.

Altered antibodies to LAMP-1, such as chimeric, humanized, CDR-grafted or bifunctional antibodies, can also be produced by methods well-known in the art. Such antibodies can also be produced by hybridoma, chemical synthesis or recombinant methods described, for example, in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual 2d ed. (Cold Spring Harbor Laboratory, 1989), incorporated herein by reference. Antibodies specific for LAMP-1 can be produced in a variety of mammals and then genetically engineered to resemble proteins of human origin and in this way avoid endogenous host defense mechanisms. Methods for "humanization" of antibodies are well-known in the art and are described, for example, in Winter and Harris, *Immunol. Today*, 14:243-246 (1993); Winter and Harris, *TIPS* 14:139-143 (1993) and Couto *et al. Cancer Res.*, 55:1717-1722 (1995).

After generating antibodies specific to LAMP-1, cytotoxic or cytostatic agents can be attached to the antibodies specific to LAMP-1 by a variety of methods known in the art. Attachment, coupling, or conjugation can be accomplished by, for example, covalent bond

formation; however, other means known in the art can be equally applied as well. Essentially any type of coupling methodology will be suitable so long as conditions are used to maintain the functions of both of the antibody and the cytotoxic or cytostatic agent. Such methods are well-known in the art. For example, the covalent bond can be formed by way of carbodiimide, glutaraldehyde, heterobifunctional cross-linkers, and homobifunctional cross-linkers. The cross-linking of proteins can additionally be accomplished by using reactive groups within the individual protein such as carbohydrate, disulfide, carboxyl or amino groups. Coupling can be accomplished by oxidation or reduction of the native protein, or treatment with an enzyme, for example.

Numerous different cytotoxic and cytostatic agents are known by those skilled in the art. Selection of which cytotoxic or cytostatic agent to attach to an antibody specific for LAMP-1 will depend on the need and will be known, or can be determined by those skilled in the art. For example, cisplatin-based regimens are utilized for ovarian, esophageal cancer, head and neck cancer, non-small cell lung cancer and testicular cancer. Therefore, if the neoplastic cell expressing LAMP-1 is testicular in origin, cisplatin may be attached to the antibody specific to LAMP-1.

In operation, the antibody specific to LAMP-1, having attached a cytotoxic or cytostatic agent, is then placed in contact with the neoplastic cells and allowed to bind LAMP-1. This targeted delivery of cytotoxic or cytostatic agent will reduce the proliferation of the neoplastic cells in the body expressing LAMP-1. Cytotoxic or cytostatic antibodies can be administered in many possible formulations, including pharmaceutically acceptable media.

EXAMPLE 4

Antigenicity Analysis of Full Length LAMP-1

Analysis of the LAMP-1 polypeptide sequence (SEQ ID NO:1) disclosed that amino acids 323-340 constitute an antigenic region. As shown in Figure 10, the region of amino acids 323-340 had an antigenicity value of greater than zero. The remaining portions of the polypeptide had an antigenicity value of less than zero.

N- and O-glycosylation site predictions were performed on the LAMP-1 polypeptide sequence. The antigenic region of amino acids 323-340 did not contain predicted N- and O-glycosylation sites. The antigenic region, SLRALQATVGNSYKCNAE (SEQ ID NO:3) is encoded by the polynucleotide region of SEQ ID NO:2 at nucleotides 1157-1210. These nucleotide positions are based on the publicly available full length sequence NM_005561.

EXAMPLE 5

Signal Sequence Identification for LAMP-1 Polypeptide

Using a signal peptide cleavage site scan, the signal peptide was identified as consisting of amino acids 1-27 of SEQ ID NO:1. The signal peptide therefore consists of the sequence MAPRSARPLLLLLPVAAARPHALSSA (SEQ ID NO:4), based on publicly available LAMP-1 sequence NP-005552.2.

Although the invention has been described with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention. It should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

CLAIMS

1. A method of diagnosing cancer, said method comprising contacting a cancer cell with an agent capable of specifically binding to LAMP-1.

2. The method of claim 1, wherein the cancer cell is a human colon cancer cell taken from a colon tissue sample.

3. The method of claim 1, wherein the cancer cell is a human colon cancer cell taken from a non-colon tissue to which the colon cancer is suspected of metastasizing.

4. The method of claim 1, wherein the cancer cell is a human lung cancer cell.

5. The method of claim 1, wherein the agent capable of specifically binding to LAMP-1 is an antibody.

6. The method of claim 5, wherein said antibody is a monoclonal antibody.

7. The method of claim 6, wherein said monoclonal antibody is generated by using cells of a tumor biopsy as an immunogen in an animal, obtaining immune cells from the animal, and generating monoclonal antibodies.

8. The method of claim 6, wherein the monoclonal antibodies are humanized.

9. The method of claim 6, wherein the antibodies are generated by immunizing an animal with a polypeptide comprising an antigenic region of LAMP-1.

10. The method of claim 9, wherein the antigenic region comprises amino acids 323-340 of SEQ ID NO:1.

11. The method of claim 9, wherein the antigenic region comprises amino acids SLRALQATVGNSYKCNAE (SEQ ID NO:3).

12. The method of claim 9, wherein the antigenic region is encoded by consecutive nucleotides 1157-1210 of SEQ ID NO:2.

13. The method of any one of claims 1-12 wherein said agent is detectably labeled.

14. A method of reducing the proliferation of a neoplastic cell, said method comprising contacting the neoplastic cell with a cytotoxic or cytostatic agent coupled to a binding agent reactive with LAMP-1.

15. The method of claim 14, comprising administering to a mammal containing a neoplastic cell population a cytotoxic or cytostatic agent coupled to a binding agent reactive with LAMP-1 that is expressed by the neoplastic cell population, wherein the cytotoxic or cytostatic binding agent is bound by the LAMP-1.

16. The method of claim 14, wherein the cytotoxic or cytostatic agent is internalized into the intracellular compartment of said neoplastic cell.

17. The method of any one of claims 14-16, wherein said neoplastic cell is a colon

cancer cell.

18. The method of any one of claims 14-16, wherein said neoplastic cell is a lung cancer cell.

19. The method of any one of claims 14-18, wherein said mammal is a human.

20. Use of an agent capable of binding to LAMP-1 in the preparation of a medicament for treating cancer in a mammal suspected of suffering from said cancer.

21. Use according to claim 20 wherein the agent is an antibody capable of specifically binding to LAMP-1.

22. Use according to claim 21 wherein the antibody is a monoclonal antibody.

23. Use according to any of claims 20-22 wherein said agent is coupled with a cytotoxic or cytostatic agent.

24. Use according to any of claims 20-22 wherein said cancer is colon cancer.

25. Use according to any of claims 20-22 wherein said cancer is metastatic colon cancer.

26. Use according to any of claims 20-22 wherein said cancer is lung cancer.

27. Use according to any of claims 20-26 wherein said mammal is a human.

28. A kit comprising an anti-LAMP-1 antibody coupled to a cytotoxic or cytostatic agent, and instructions for administering the anti-LAMP-1 antibody coupled to a cytotoxic or cytostatic agent, to a mammal suffering from cancer.

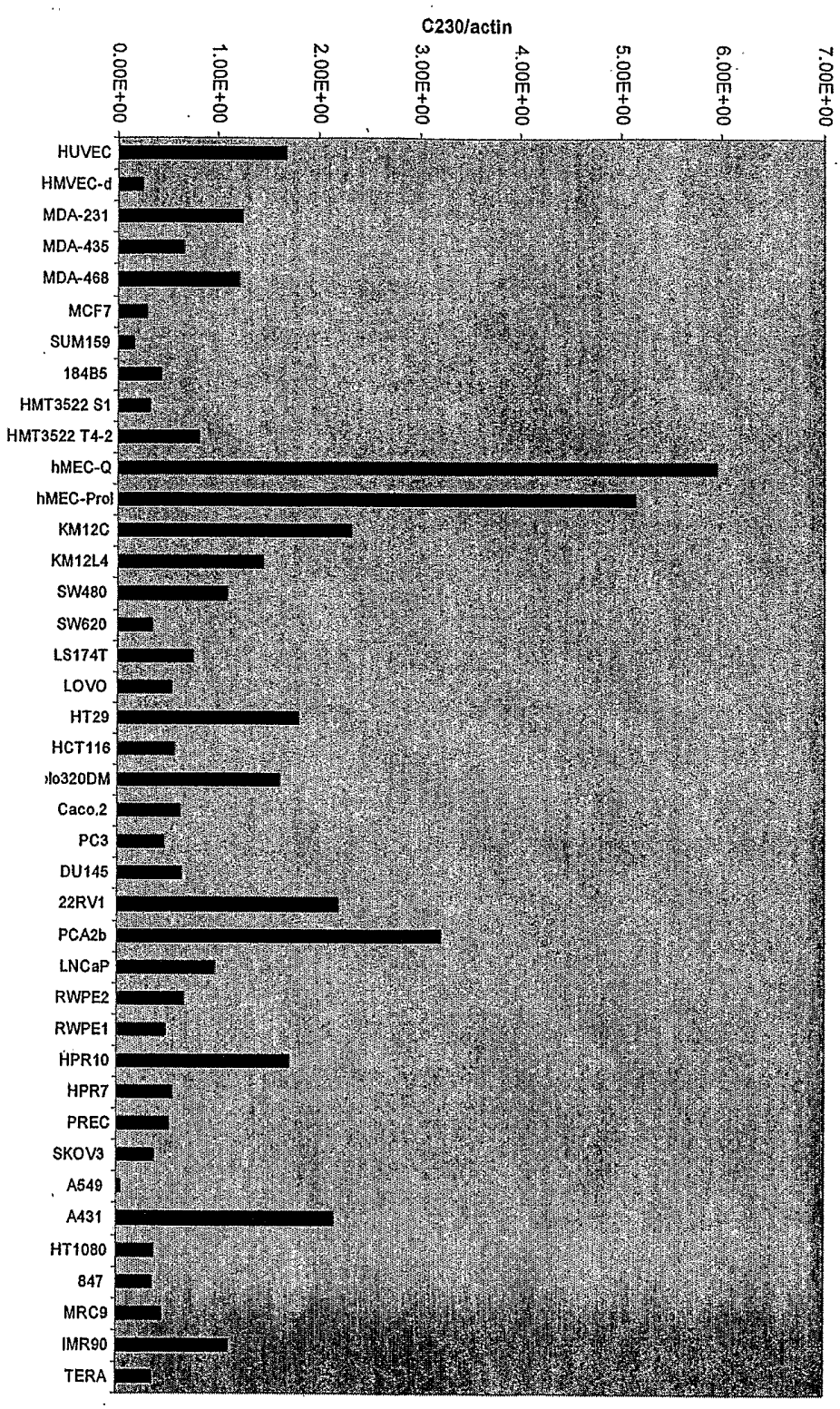


Figure 1. Levels of LAMP-1 mRNA in tumor cell lines normalized to actin

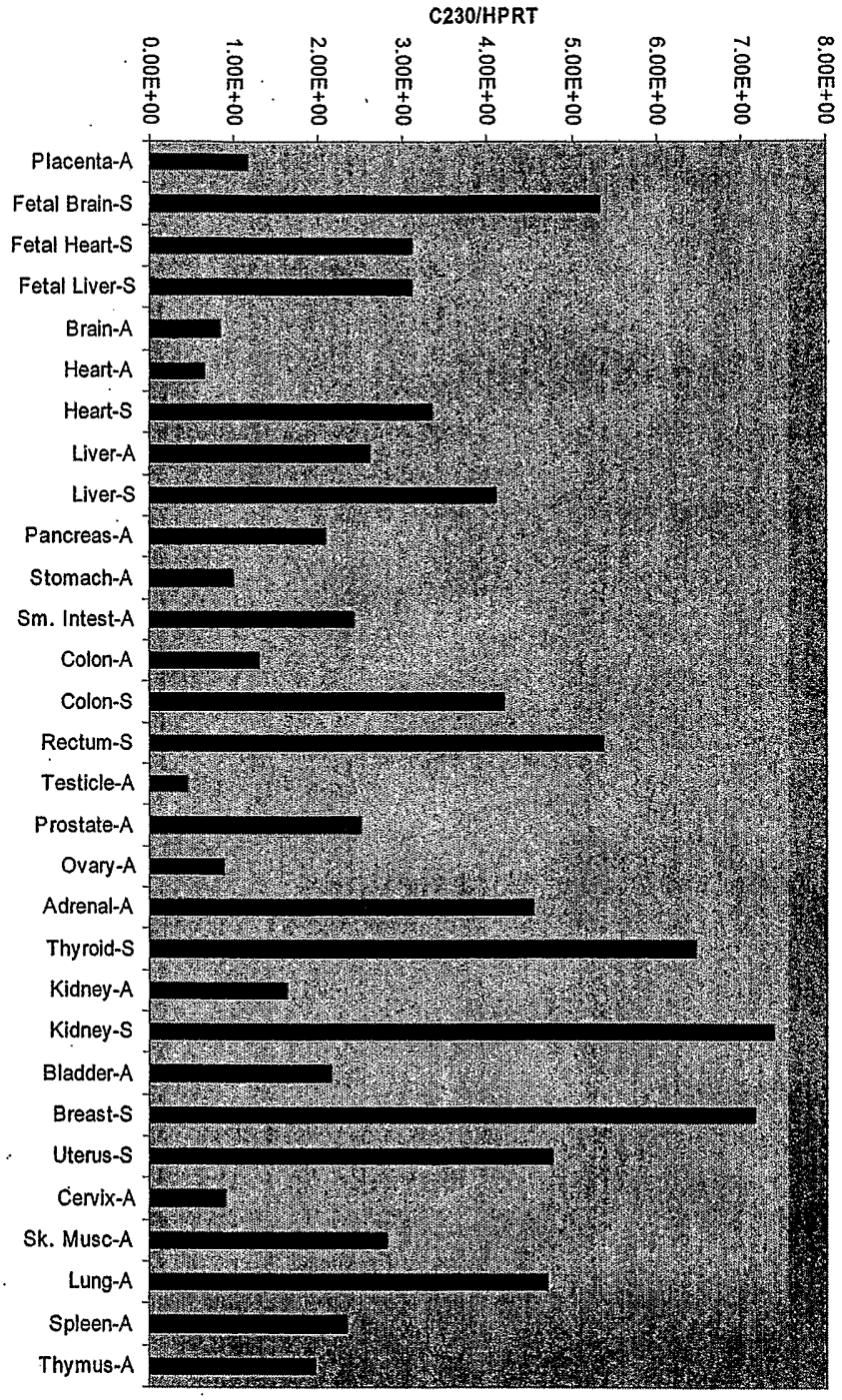
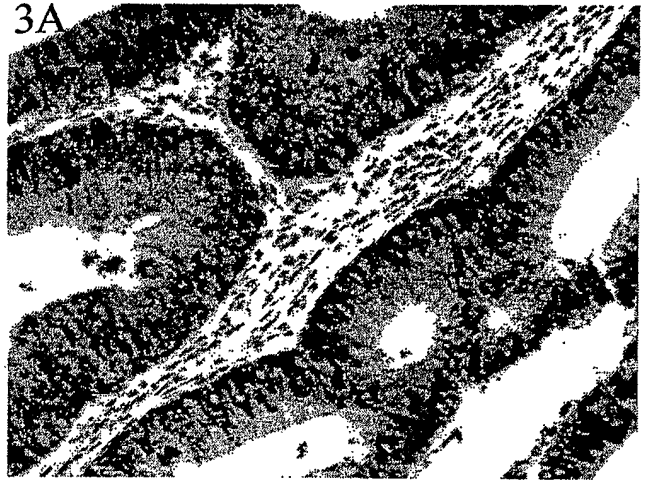


Figure 2. Levels of LAMP-1 mRNA normalized to HPRT

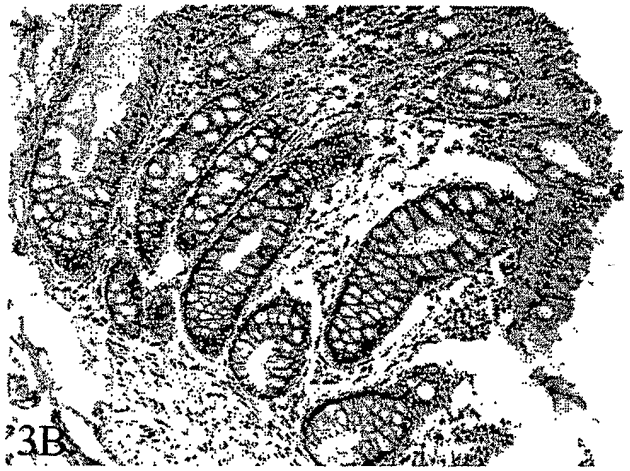
Figure 3. IHC analysis of LAMP-1 in normal colon and cancer

CC

3A

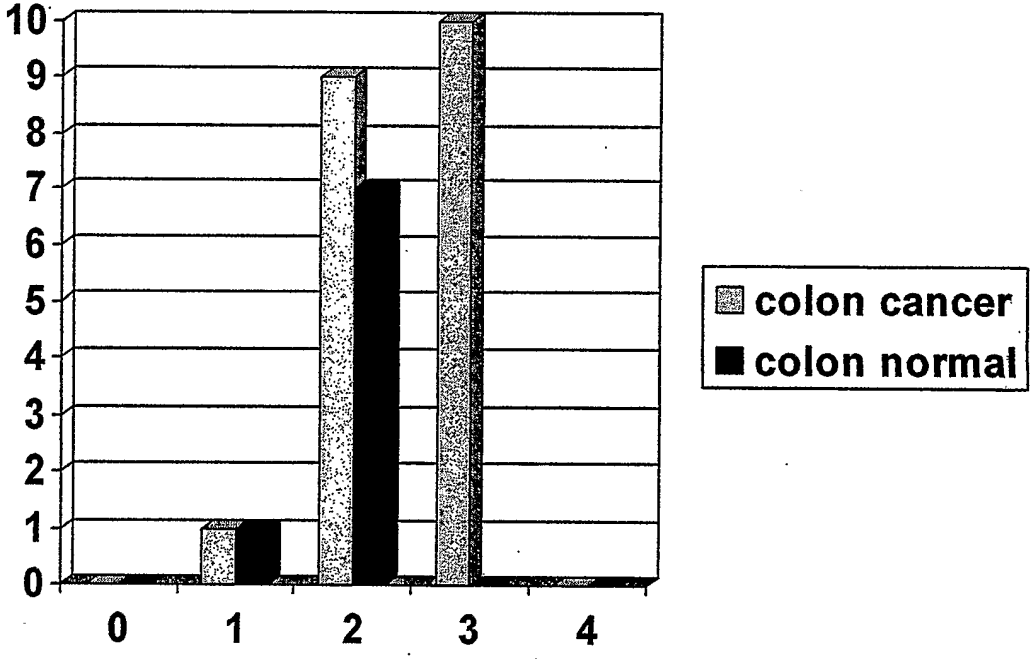


CN



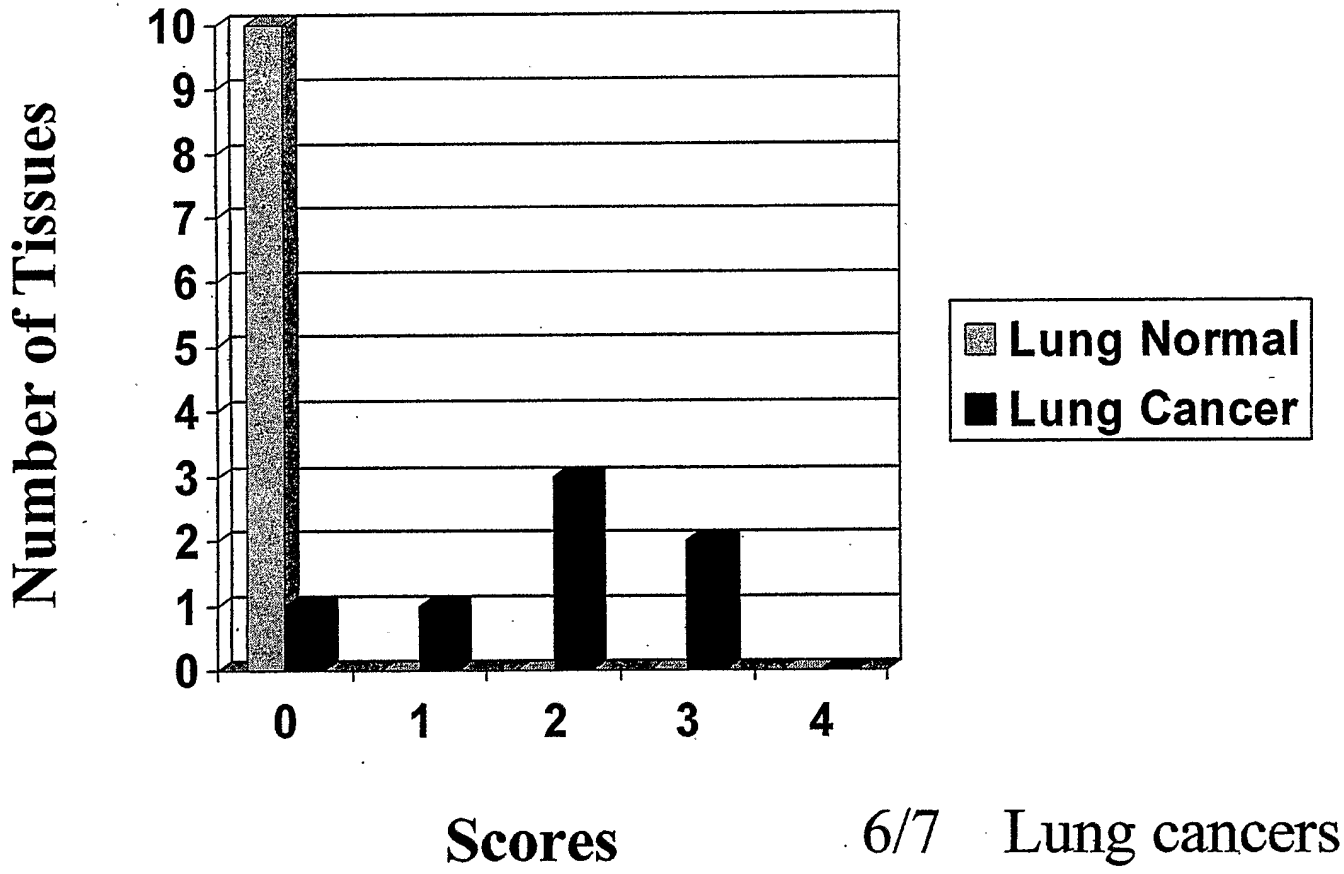
3B

3C



30/30 colon cancers stained
8/8 colon normals stained

Figure 4. IHC analysis of LAMP-1 in lung tissue

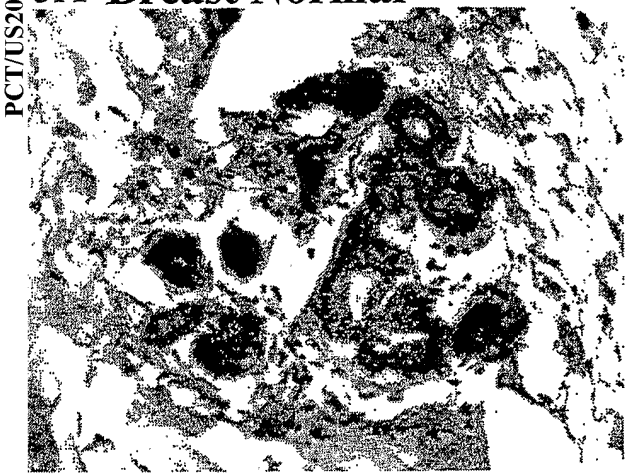


6/7 Lung cancers stained

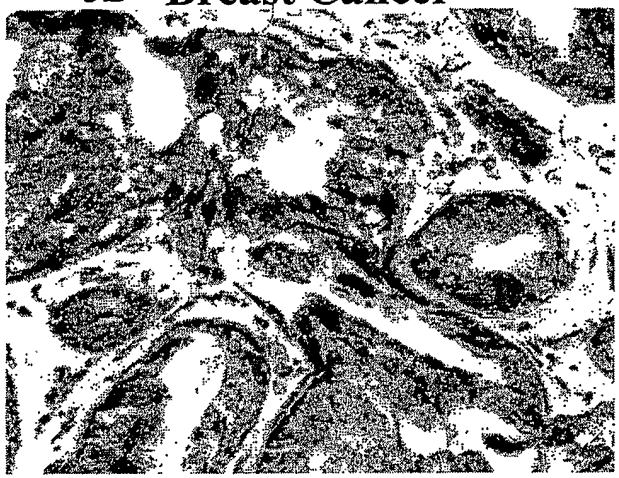
0/10 Lung normals stained

Figure 5. IHC analysis of LAMP-1 in breast & prostate

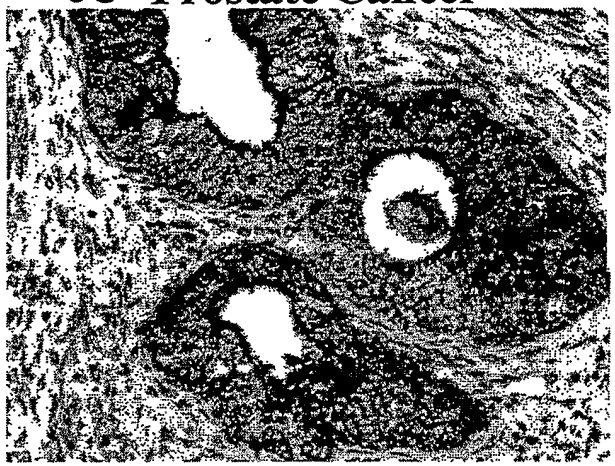
5A Breast Normal



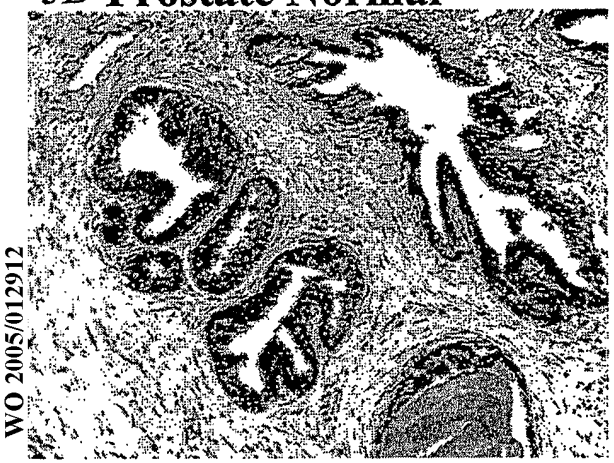
5B Breast Cancer



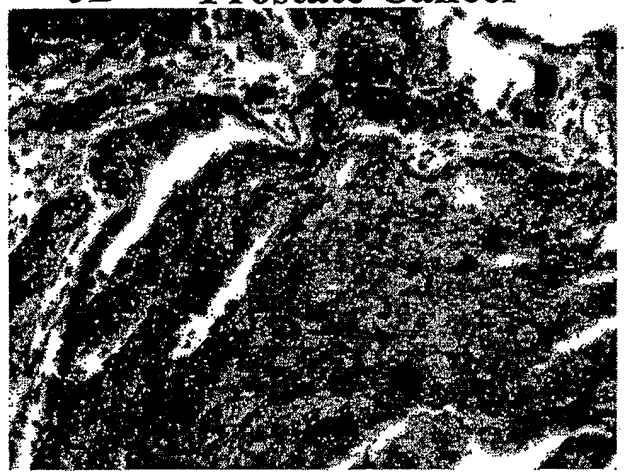
5C Prostate Cancer



5D Prostate Normal



5E Prostate Cancer



5F Prostate Cancer

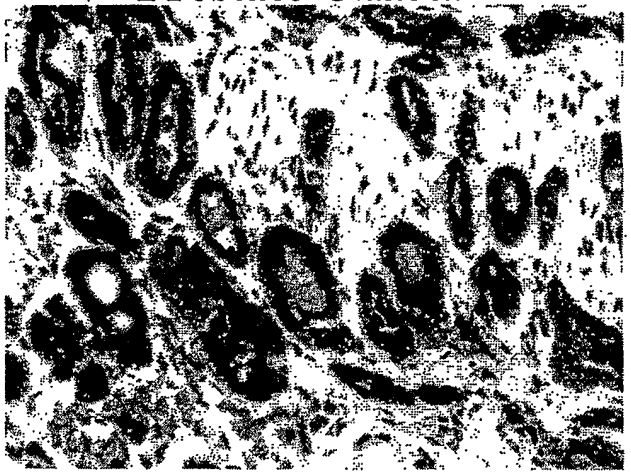
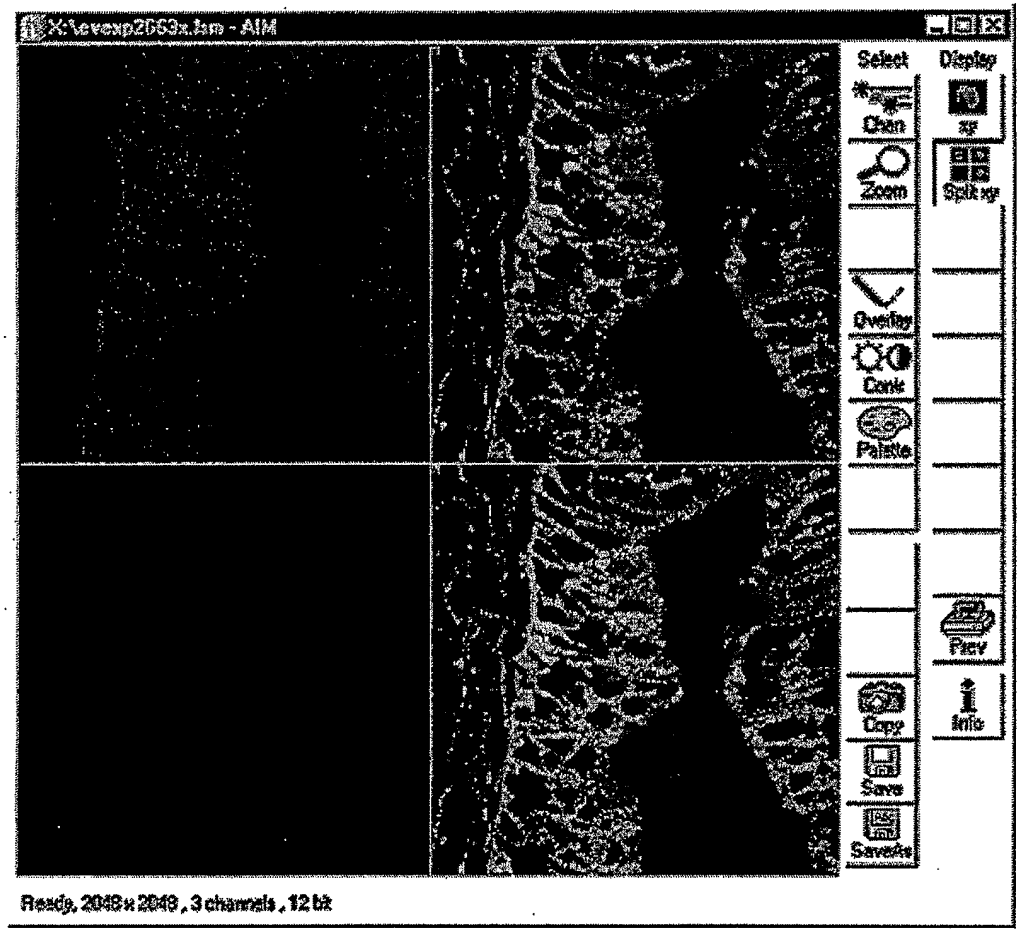


Figure 6. Colocalization of LAMP-1 with surface membrane marker CD44 in human colon cancer but not in normal colon tissue

CD44

LAMP-1

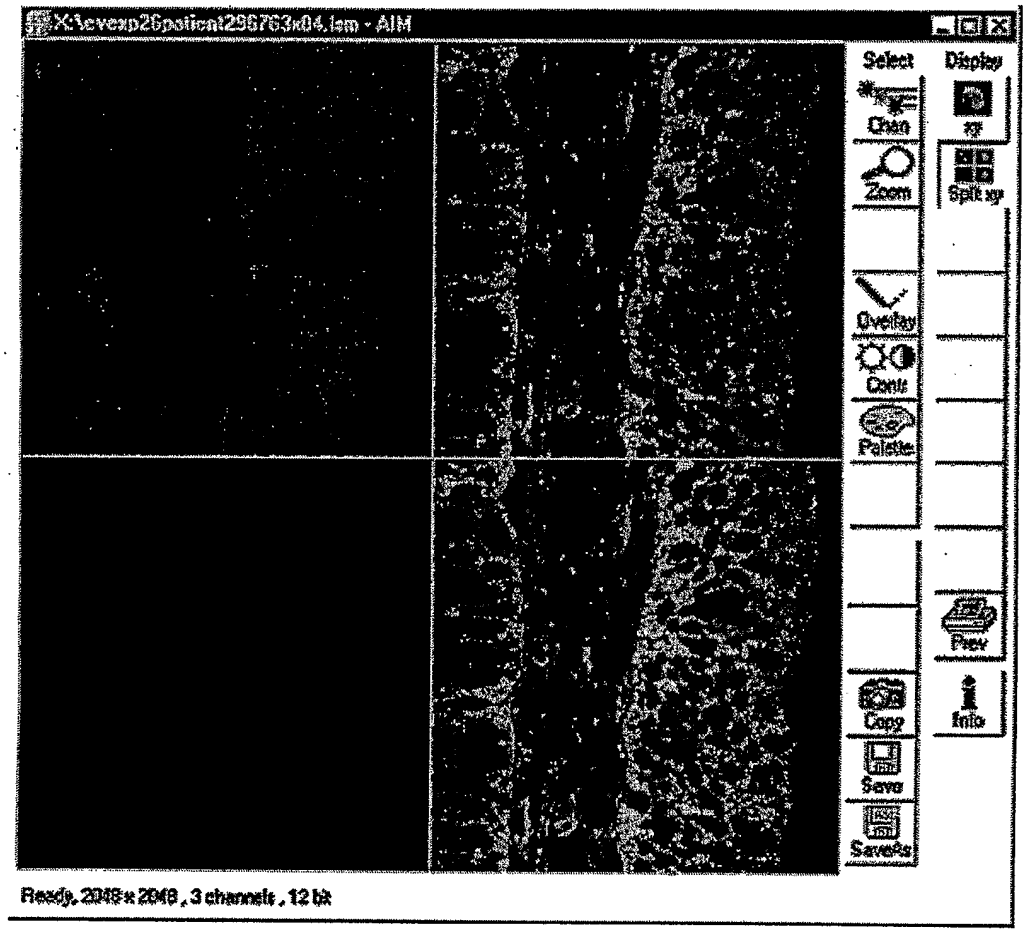


Merged image

Human colon cancer

Figure 7. Colocalization of LAMP-1 with surface membrane marker CD44 in human colon cancer but not in normal colon tissue

CD44



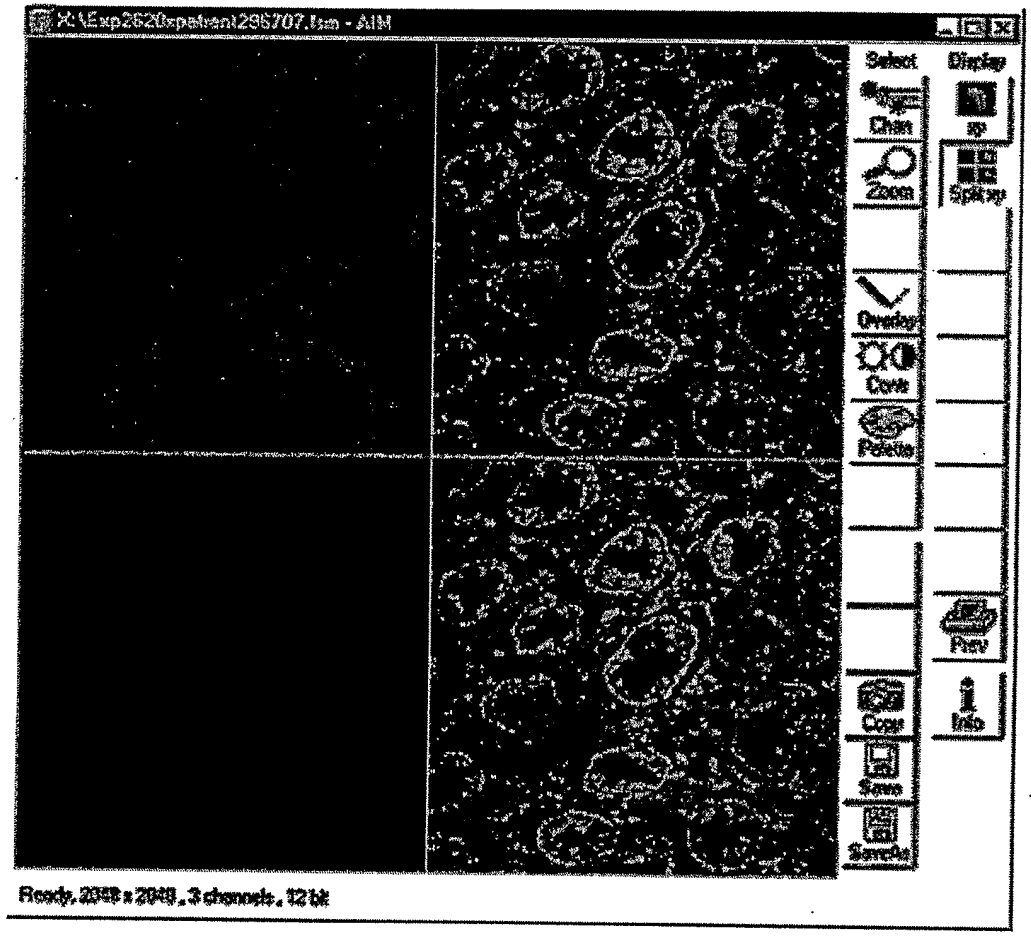
LAMP-1

Merged image

Human colon cancer

Figure 8. Colocalization of LAMP-1 with surface membrane marker CD44 in human colon cancer but not in normal colon tissue

CD44



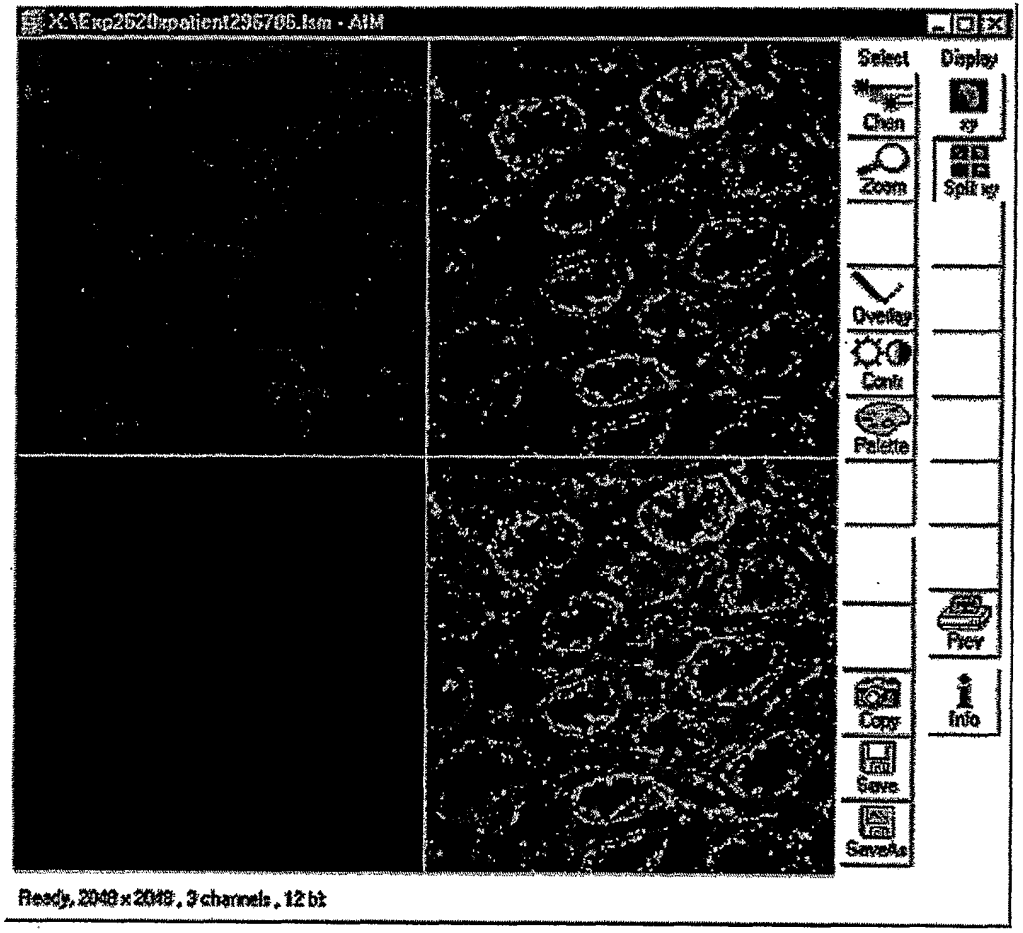
LAMP-1

Area of normal tissue within frozen tissue section of colon cancer: distinct lack of colocalization of *LAMP-1* with CD44

Merged image

Figure 9. Colocalization of LAMP-1 with surface membrane marker CD44 in human colon cancer but not in normal colon tissue

CD44



LAMP-1

Area of normal tissue within frozen tissue section of colon cancer: distinct lack of colocalization of LAMP-1 with CD44

Merged image

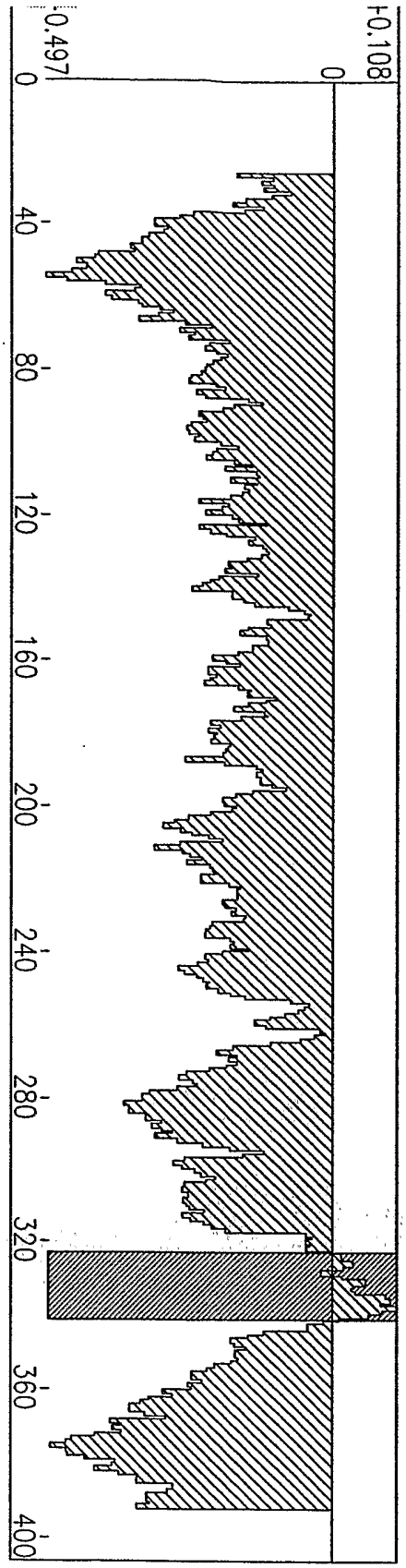


FIG.10

Signal_1
1 MAPRSARRPL LLLLPVAAAR PHALSSAAMF MVKNGNGTAC IMANFSAAFS
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101 LNFTRNATRY SVQLMSFVYN LSDTHLFPNA SSKEIKTVES ITDIRADIDK
151 KYRCVSGTQV HMNNVTVTLH DATIQAYLSN SSFSRGETRC EQDRPSPTTA
201 PPAPPSPSPS PVPKSPSVDK YNVSGTNGTC LLASMGLQLN LTYERKDNTT
251 VTRLLNINPN KTSASGSCGA HLVTLELHSE GTVLLFQFG MNASSSRFFL
301 QGIQLNTILP DARDPAFKAA NGSIRALQAT VGMSYKCMEG EHVRVTKAFS
351 VNIFKVWVQA FKVEGGQFGS VEECLLDENS TLIPIAVGGA LAGLVLIVLI
401 AYLVGRKRSH AGYQTI

FIG. 11

SEQ ID NO:1

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  61 taacgcgctg tctctaacgc cagcgccgtc tcgcgcgcac tgcgcacaga ccacccgcag
 121 acgcccggca gtccgcaggc ccaaacgcgc acgcgacccc gctctccgca ccgtacccgg
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