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(19) **United States**(12) **Patent Application Publication**
Awasthi et al.(10) **Pub. No.: US 2014/0065207 A1**(43) **Pub. Date: Mar. 6, 2014**(54) **THERAPIES FOR CANCER USING RLIP76**(71) Applicant: **Board or Regents, The University of Texas System**, Austin, TX (US)(72) Inventors: **Sanjay Awasthi**, Arlington, TX (US);
Sharad S. Singhal, Arlington, TX (US);
Sushma Yadav, Arlington, TX (US)(73) Assignee: **Board or Regents, The University of Texas System**, Austin, TX (US)(21) Appl. No.: **14/055,739**(22) Filed: **Oct. 16, 2013****Related U.S. Application Data**

(60) Division of application No. 12/055,138, filed on Mar. 25, 2008, now Pat. No. 8,586,553, which is a continuation of application No. 11/264,910, filed on Nov. 2, 2005, now abandoned, which is a continuation-in-part of application No. 10/714,506, filed on Nov. 13, 2003, now abandoned, which is a continuation-in-part of application No. 10/713,578, filed on Nov. 13, 2003, now abandoned.

(60) Provisional application No. 60/425,917, filed on Nov. 13, 2002, provisional application No. 60/425,814, filed on Nov. 13, 2002.

Publication Classification(51) **Int. Cl.****A61K 39/395** (2006.01)**C12N 15/113** (2006.01)(52) **U.S. Cl.**CPC **A61K 39/3955** (2013.01); **C12N 15/1138** (2013.01)USPC **424/450**; 435/375; 424/172.1; 424/139.1; 514/44 A(57) **ABSTRACT**

The present invention is a composition identified as a region of ralA binding protein **1**, wherein the region neighbors a membrane-associated portion of the ralA binding protein **1**, reduces transport activity and membrane association of the ralA binding protein **1** and kills cells undergoing uncontrolled cell growth in a subject that has cells undergoing uncontrolled cell growth. The region is used to generate medicines that kill malignant cells and tumorigenic cells. Medicines may be in the form of antibodies, si-RNA and small molecules that recognize the region.

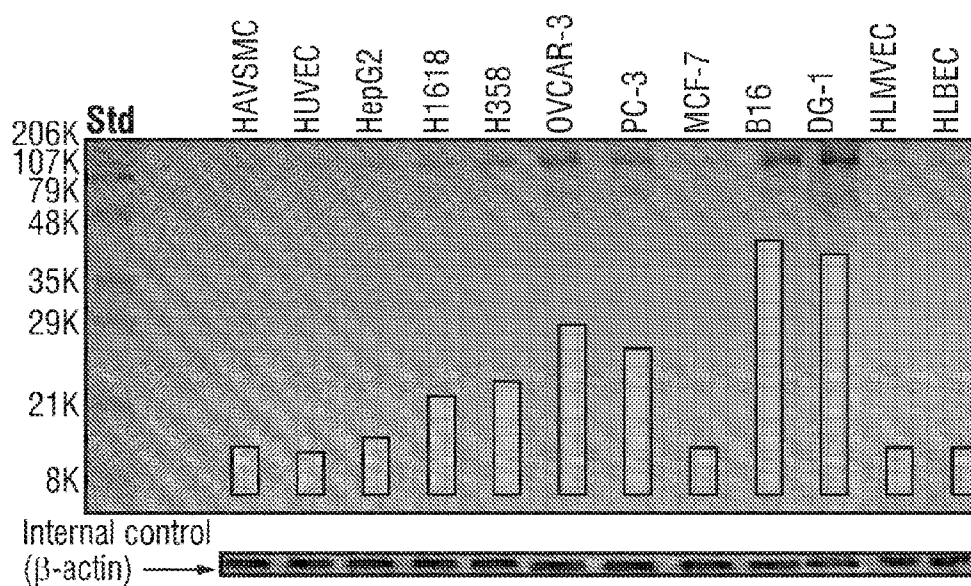


FIG. 1

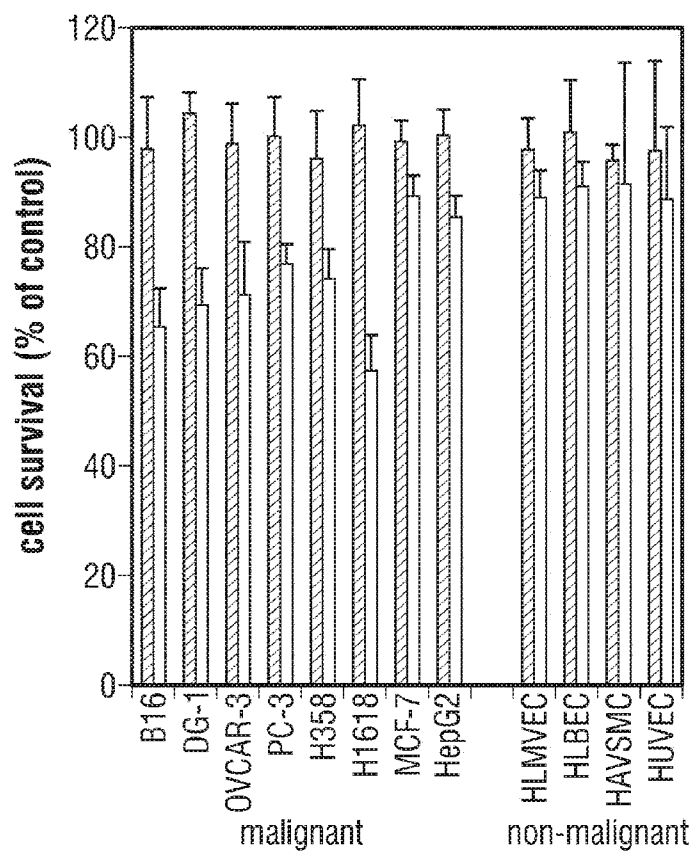
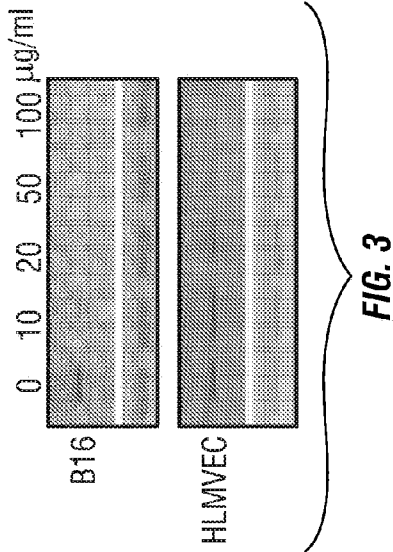
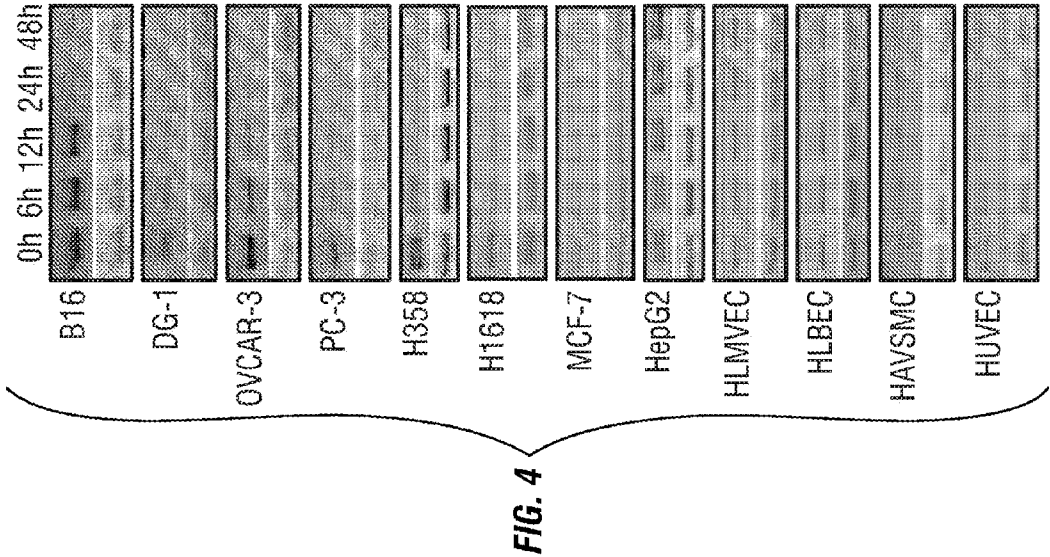
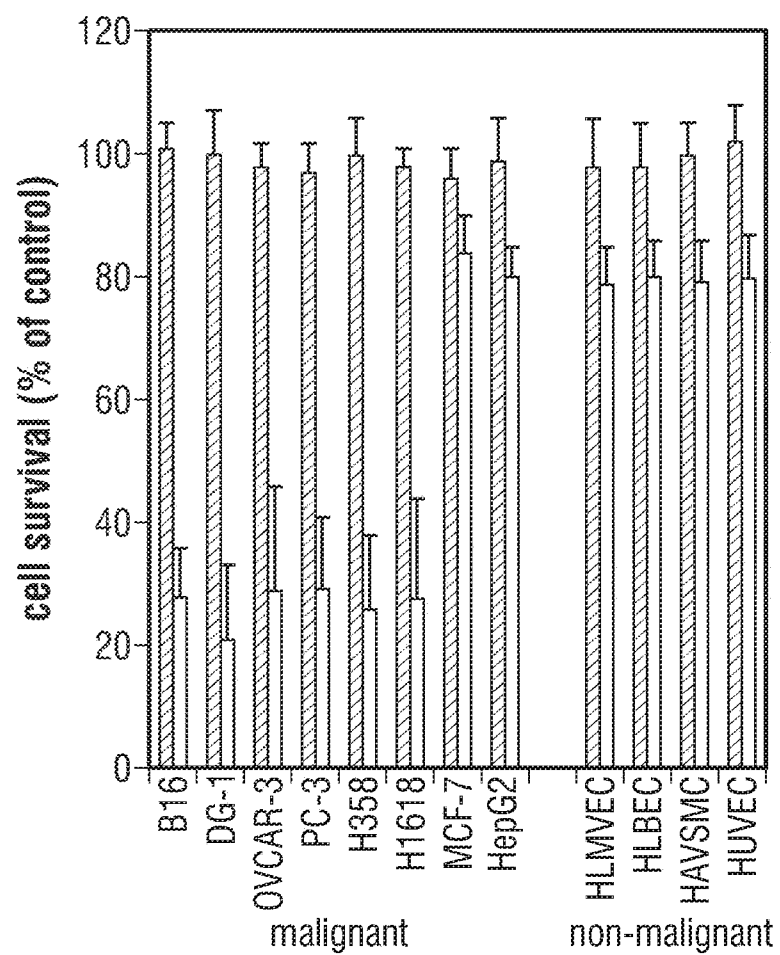
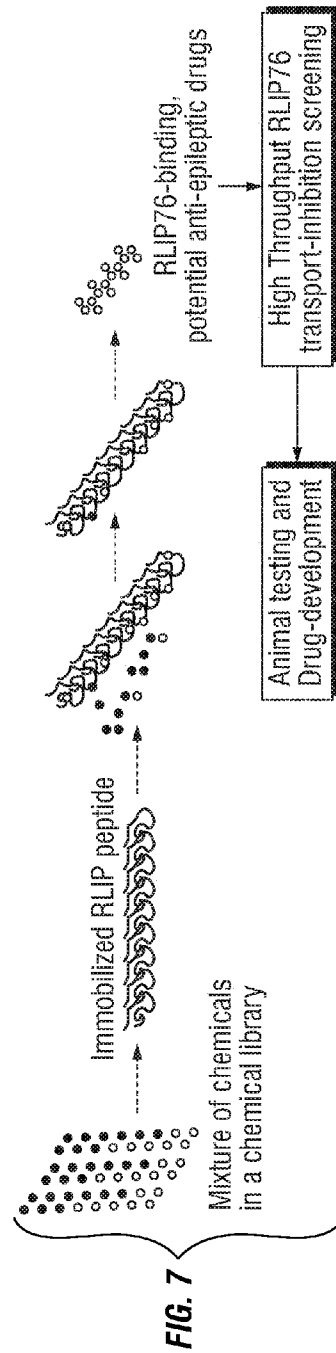
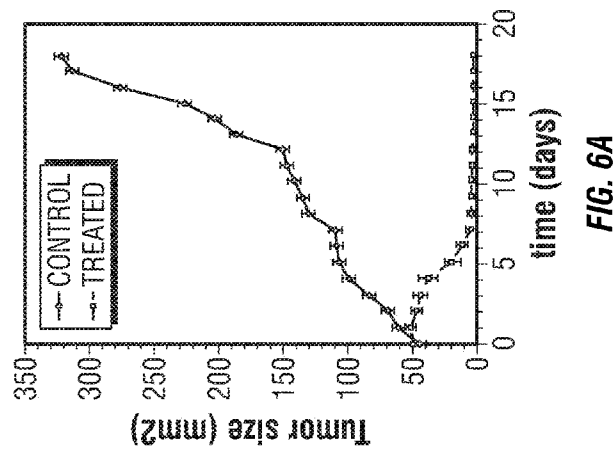
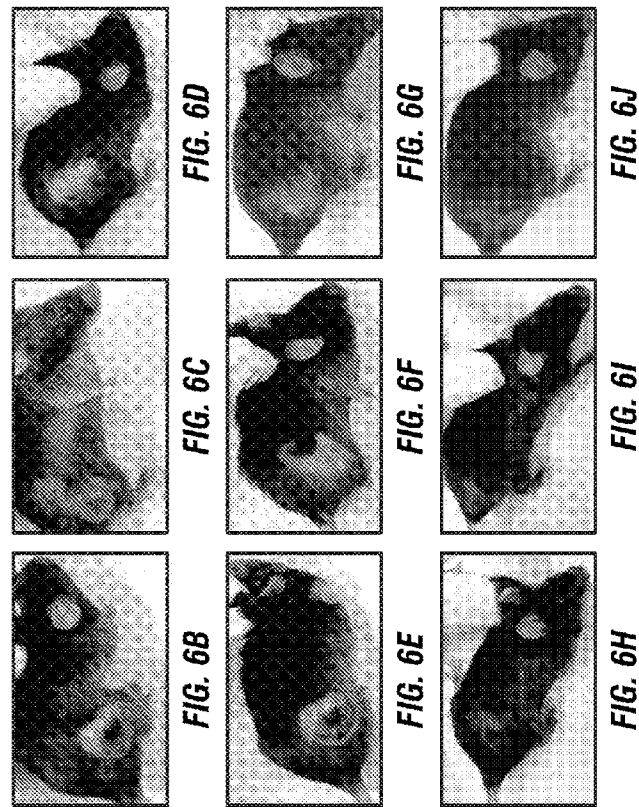


FIG. 2



**FIG. 5**



THERAPIES FOR CANCER USING RLIP76

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 11/264,910, filed Nov. 2, 2005, which is a continuation-in-part of prior U.S. application Ser. No. 10/714,506, filed Nov. 13, 2003, which claims the benefit of U.S. Provisional Patent Application No. 60/425,917, filed Nov. 13, 2002, all of which are incorporated herein by reference for all purposes. This application is also a continuation-in-part of prior U.S. application Ser. No. 10/713,578, filed Nov. 13, 2003, which claims the benefit of U.S. Provisional Patent Application No. 60/425,814, filed Nov. 13, 2002, both of which are incorporated herein by reference for all purposes.

STATEMENT REGARDING FEDERALLY SPONSORED APPLICATIONS

[0002] The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant No. NIH 2-R01-CA77495, Grant No. NIH CA 104661.

REFERENCE TO A SEQUENCE LISTING

[0003] This application incorporates by reference sequence listing material included on computer readable form and identified as 124263-1040 RLIP.ST25.txt saved on Nov. 2, 2005, in ASCII readable form.

BACKGROUND OF THE INVENTION

[0004] The present invention relates to improved anti-cancer therapies, particularly one targeted towards cells expressing high levels of a ral interacting protein, RLIP76.

[0005] Current therapies for human cancers are generally unsatisfactory because of a high rate of failure and excessive side-effects. Side effects are generally significant and result, in part, because few current therapies are targeted to cancerous cells alone. There remains a need to identify targeted therapies that eliminate only cancerous cells and not healthy cells.

SUMMARY OF THE INVENTION

[0006] The present invention solves problems associated with current therapies for cancerous cells or those that undergo uncontrolled cell growth (e.g., cancerous, tumorigenic and malignant cells). In particular, the present invention provides compositions for inhibiting and/or depleting RLIP76.

[0007] As provided herein, RLIP76 is a membrane associated protein and a critical as well as predominant regulator of transport in cancerous cells or those undergoing uncontrolled cells growth. Targeting therapies against RLIP76 directly effects RLIP76 transport activity and reduces the number and/or potency of such cells undergoing uncontrolled cell growth in subjects having such uncontrolled and growing cells.

[0008] The present invention provides for compositions that target RLIP76 and reduce the number and/or potency of cells undergoing uncontrolled cell growth. Compositions comprise a region homologous to a portion of a ralA binding

protein **1**, wherein the region neighbors a membrane-associated portion of the ralA binding protein **1**, reduces transport activity and membrane association of ralA binding protein **1** and kills cells undergoing uncontrolled cell growth in a subject that has cells undergoing uncontrolled cell growth.

[0009] Compositions of the present invention include an internal peptide region of RLIP76 to be used as bait in screens of chemical libraries for synthetic and naturally occurring organic chemicals and compounds with an ability to reduce the number and/or potency of cells undergoing uncontrolled cell growth. The identified chemicals and compounds are those acting as specific inhibitors of RLIP76 activity (e.g., found to reduce the number and/or potency of cells undergoing uncontrolled cell growth). As such, the present invention provides for compositions that are improved therapies for cancers, malignancies, and tumorigenic or uncontrolled growth cells that express RLIP76.

[0010] In one form, the present invention provides for a region recognizing a ralA binding protein **1**, wherein the region further comprises SEQ ID NO:28 and modified variants thereof.

[0011] In other forms, the present invention provides for compositions and methods of using such compositions as SEQ ID NO: 3 to SEQ ID NO:19 and SEQ ID NO:21. Such compositions, in various forms to be used to identify compounds as medicines for cancers, malignancies and tumors and for reducing the number and/or potency of cells undergoing uncontrolled cell growth.

[0012] Those skilled in the art will further appreciate the above-noted features and advantages of the invention together with other important aspects thereof upon reading the detailed description that follows in conjunction with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] For more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying figures, wherein:

[0014] FIG. 1 depicts comparison of RLIP76 levels in cultured malignant vs. non-malignant cells;

[0015] FIG. 2 depicts effects of pre-immune IgG (dark bars) and anti-RLIP76 IgG (white bars) (37 µg/ml final) on cell survival as determined by MTT assay;

[0016] FIG. 3 depicts a depletion of RLIP76 expression in malignant and non-malignant cells using RLIP76 si-RNA;

[0017] FIG. 4 depicts a time dependent effect of RLIP76 si-RNA in malignant and non-malignant cells;

[0018] FIG. 5 depicts MTT assays for malignant and non-malignant cells performed 48 hours after treatment with si-RNA in which grey bars show scrambled si-RNA white bars show RLIP76 si-RNA; and

[0019] FIGS. 6 A and B-J depict antineoplastic effects of RLIP76 inhibition or depletion in mouse melanoma in which (A) shows tumor measurements pooled from control animals, including PBS, pre-immune IgG, scrambled siRNA antisense injection (circle), versus treated animals, including anti-RLIP76 IgG, RLIP76 siRNA, RLIP76 phosphorothioate antisense injection (square), and (B)-(J) show photographs of representative animals taken at eight days after treatment.

[0020] FIG. 7 is a diagram of a method of identifying inhibitors or RLIP76 transport activity.

DETAILED DESCRIPTION OF THE INVENTION

[0021] Although making and using various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many inventive concepts that may be embodied in a wide variety of contexts. The specific aspects and embodiments discussed herein are merely illustrative of ways to make and use the invention, and do not limit the scope of the invention.

[0022] In the description which follows like parts are marked throughout the specification and drawing with the same reference numerals, respectively. The drawing figures are not necessarily to scale and certain features may be shown exaggerated in scale or in a somewhat generalized or schematic form in the interest of clarity and conciseness.

[0023] The following are abbreviations that may be used in describing the present invention: RLIP, ral interacting protein; MDR, multi-drug resistance; GS-E, glutathione-electrophile conjugates; phenyloin, PHE; carbamazepine, CBZ.

[0024] Current antineoplastic therapies for human malignancies are limited by the occurrence of significant normal tissue toxicities due to inherent relatively non-specific genotoxic or signaling effects. Attempts to improve antineoplastic therapies have focused on identifying targets which are preferentially expressed in cancer cells, which when inhibited cause apoptosis in malignant cells while sparing cells of normal tissues. Delineation of differentially expressed signaling proteins that are responsible for un-regulated growth and suppression of normal apoptotic pathways has led to the identification of numerous potential targets. While new agents have emerged, such as antibodies including rituximab (anti-CD20 antibody for lympho-proliferative disorders), trastuzumab (anti-Her-2/neu antibody for breast cancer) and small molecules including imatinib mesylate (Bcr-Abl kinase inhibitor for chronic myelogenous leukemia) and erlotinib (tyrosine kinase inhibitor for a variety of solid tumors), their overall efficacy remains limited, because of their limited effectiveness in only a small fraction of patients with such malignancies or because of clonal selection of cancer cells inherently refractory to such therapies. Thus, development of new targeting molecules and discovery of new targets remains critical.

[0025] RLIP76 (also referred to herein as RALBP1) represents a novel target for cancer therapy not only because of its involvement in the rate-controlling step in glutathione (GSH)-mediated metabolism of electrophilic or oxidant chemicals often used as anti-neoplastic agents, but also because of its apparent linkage to key signaling pathways known to be crucial for the survival, proliferation, and motility of malignant cells. The present inventors have recently demonstrated that lack of RLIP76 in knockout mice leads to loss of nearly 4/5 of total GSH-conjugate (GS-E) as well as anthracycline-transport activity, and widespread changes in GSH-linked antioxidant enzymes (as disclosed, e.g., in Awasthi S, et al. *Cancer Res* 2005; 65:6022-8). RLIP76^{-/-} mice develop a characteristic sensitivity to stress, particularly to ionizing radiation. These and other studies by the inventors in cell culture systems implicate RLIP76 as a part of stress-defenses (as disclosed, e.g., in Yang, et al., *J Biol Chem* 2003; 278:41380-88). In particular, the inventors have determined that inhibition of RLIP76 transport function using antibodies to a cell-surface epitope or depletion of RLIP76 using si-RNA uniformly causes apoptosis in a variety of histological types of cancers (as disclosed, e.g., in Awasthi, et al., *Int J Oncol* 2003; 22:713-20; Awasthi, et al., *Int J Oncol* 2003; 22:721-32;

Yadav, et al., *Biochemistry* 2004; 43:16243-53; Struckler, et al., *Cancer Res* 2005; 65:991-8).

[0026] [00261] The present invention provides clinical applicability of RLIP76 as a target for anti-cancer therapy. The present invention confirms that there is a greater dependence on RLIP76 in cancer cells, due to greater expression of the protein in such cells. The present invention shows that this dependence translates to a greater susceptibility of cancer cells to agents that deplete RLIP76; hence, RLIP76 is a powerful target a broad-spectrum anti-cancer therapy.

[0027] Anti-RLIP agents, those that reduce and inhibit protein function or deplete expression of the protein are provided herein. Such agents include anti-RLIP76 IgG, si-RNA and anti-sense DNA oligonucleotides. With the present invention, it is demonstrated that because RLIP76 is expressed to a greater degree in malignant cells and RLIP76 inhibition or depletion causes preferential toxicity towards malignant cells, anti RLIP agents exert significant antineoplastic effects in the malignant cells.

[0028] The inventors, Sanjay Awasthi and Sharad S. Singhal, of the present invention have recently described a novel non-ABC transporter that appears to be multispecific as a Ral interacting protein (see U.S. application Ser. No. 10/714,506; U.S. application Ser. No. 10/713,578; each incorporated herein by reference). As used herein, this transporter is referred to RLIP76 or RalBP1. The official human genome name for the protein is RALBP1 (SEQ ID NO:1; and SEQ ID NO:22 for the coding sequence). RLIP76 is a modular multifunctional and modular protein found ubiquitously in many species from *Drosophila* to humans. It is encoded in humans on chromosome 18p11.3 by a gene with 11 exons and 9 introns. The protein product, also known as ralA binding protein 1, is typically a 76 kDa (SEQ ID NO:2; and SEQ ID NO:23 for the coding sequence) protein; however, splice-variants including a 67 kDa peptide and a longer 80 kDa or 102 kDa peptide, cytoctrin, have also been identified.

[0029] Malignant cells contain a greater quantity of antigenically detectable RLIP76 as shown in the Table and in FIG. 1 in which RLIP76 obtained from various cell lines (of different origin) was quantitated (not all purification data shown). The Table and FIG. 1 shows there to be greater RLIP76 in malignant cells as compared with non-malignant cells. Total RLIP76 protein was purified from membrane fraction of several malignant cell lines, including human small cell lung cancer (SCLC; H1618), non-small cell lung cancer (NSCLC; H358), ovarian (OVCA3-3), breast (MCF-7), prostate (PC-3), liver (HepG2), melanoma (DG-1), mouse melanoma (B16-F1) and non-malignant human cell lines of endothelial (HUVEC, HLMVEC), epithelial (HLBEC), and aortic smooth muscle (HAVSMC) origin. Purification folds of 120 to 153 were observed, and single protein band of intact RLIP76 were seen in SDS PAGE, which were recognized by purified polyclonal rabbit-anti-human RLIP76 antibodies in Western blot analyses. Antibodies were not non-cross-reactive with other proteins including PgP or MRP-1. No significant contamination was observed in the Western blots. Representative purified protein data, quantified by ELISA, are shown in TABLE 1. Western blot analyses of crude membrane fraction from each cells with lanes loaded with equal amount of crude protein (200 µg) are shown in FIG. 1. RLIP76 was purified by DNPSG-affinity as described previously by the inventors (Singhal, et al., *Int J Oncol* 2003; 22:365-75). Briefly, cells were pelleted and washed with a balanced salt solution (138 mM NaCl, 5 mM KCl, 0.3 mM

KH₂PO₄, 0.3 mM Na₂HPO₄, 4 mM NaHCO₃, and 5.6 mM glucose, pH 7.4). Cells were lysed in a lysis buffer (10 mM Tris-HCl, pH 7.4, containing 1.4 mM BME, 0.1 mM PMSE, 0.05 mM BHT and 0.1 mM EDTA) followed by sonication and recovery of pellets in lysis buffer containing 0.25% polidocanol. After incubation for 4 hours in the cold, samples were centrifuged and supernatants applied to DNP-SG Sepharose-affinity column. Homogenous RLIP76 was obtained using methods known to one of ordinary skill in the art.

TABLE

RLIP76 Protein and transport activity in malignant and non-malignant cell lines.				
	RLIP76 Protein		Transport Activity	
	μg/10 ⁸	% of total	(pmol/min/mg)	
	cells	protein	DOX	DNP-SG
MALIGNANT CELLS				
B16 (mouse melanoma)	71 ± 6	0.8	443 ± 34	1796 ± 145
DG-1 (human melanoma)	63 ± 5	0.8	384 ± 28	1562 ± 112
OVCAR-3 (human ovary)	53 ± 3	0.7	298 ± 23	1194 ± 122
PC-3 (human prostate)	46 ± 3	0.6	211 ± 26	893 ± 66
H358 (human lung, NSCLC)	36 ± 3	0.6	180 ± 15	695 ± 56
H1618 (human lung, SCLC)	32 ± 3	0.5	96 ± 12	361 ± 41
MCF-7 (human breast)	15 ± 1	0.2	36 ± 3	105 ± 7
HepG2 (human liver)	17 ± 1	0.3	55 ± 5	167 ± 14
NON-MALIGNANT CELLS				
HLMVEC (human lung endothelium)	19 ± 2	0.3	40 ± 5	136 ± 14
HLBEC (human lung epithelium)	22 ± 2	0.4	46 ± 4	150 ± 20
HAVSM (human aorta smooth muscle)	15 ± 1	0.3	40 ± 6	125 ± 10
HUVEC (human umbilical endothelial)	14 ± 1	0.2	36 ± 4	113 ± 12

[0030] Transport activity of RLIP76 is greater in malignant cells as determined by membrane vesicles prepared from plasma membrane fractions from each cell line, enriched for inside-out vesicles (IOVs) by wheat-germ agglutinin affinity chromatography as previously described by the inventors (see, e.g., Awasthi, et al., Int J Cancer 2004; 112:934-42). Results of measurements of ATP-dependent transport of ¹⁴C-doxorubicin (DOX) as well as ³H-dinitrophenyl S-glutathione (DNP-SG) using a standardized 96 well-plate transport assay revealed greater transport of both substrates in cells containing greater amount of RLIP76 protein. The Table shows the general correlation between RLIP76 protein level and transport activity. The greatest transport activity was found in melanoma cells with transport activity greater than in malignant than non-malignant cells. Low expression of RLIP76 in MCF-7 and HepG2 correlated with lower transport rate in the crude-membrane vesicles. Total DOX or DNP-SG transport rate correlated with either RLIP76 protein amounts (Table) or RLIP76 ATPase activity (data not shown). ATPase activity was measured using aliquots of protein fraction containing 1 to 10 μg protein added to a 0.5 mL reaction mixture (50 mM Tris-HCl pH 7.4, 10 mM MgCl₂, 2 mM EGTA, 0.8 mM sodium phosphate, 2.8 mM β-mercaptoethanol, and 1 mM ouabain) that incubated for 5 minutes at 37° C. Each

reaction was initiated with 1.6 mM γ-³²P-ATP with or without 0.12 mM DNP-SG or 0.01 mM DOX. After 60 minutes each reaction was terminated by addition of a 2.5 ml cold mixture of 1 M perchloric acid and 5% ammonium molybdate in water (4:1) followed by extraction with a 2.5 mL mixture of isobutanol-benzene (1:1). Radioactivity was quantified in the organic phase to determine cleaved terminal phosphate. RLIP76 activity was calculated by subtracting background counts obtained in the absence of protein from those obtained in the presence of protein. RLIP76 activity was calculated by subtracting the activity seen in the absence of DOX or DNP-SG from that seen in its presence. Each assay was performed in triplicate.

[0031] Crude membrane vesicles (inside-out vesicles or IOVs) were prepared from non-malignant cells (HAVSMC, HLBEC, HLMVEC and HUVEC) and cancer cells (SCLC, NSCLC, DG-1 human melanoma, mouse B16 melanoma, MCF7, HepG2, PC-3 and OVCAR-3) identified in the Table using procedures known in the art (see, e.g., Awasthi, et al., Clin Invest 1994; 93:958-65; Awasthi, et al., Biochemistry 2000; 39:9327-34). Transport studies in IOVs were performed by method known in the art (see, e.g., Awasthi, et al., Int J Cancer 2004; 112:934-42) in which heat-inactivated IOVs were used as negative controls.

[0032] RLIP76 inhibition or depletion caused preferential cytotoxicity in malignant cells. RLIP76 was inhibition by anti-RLIP76 IgG or via depletion using si-RNA (si-RNA⁵⁰⁸⁻⁵²⁸) targeted to a specific identified region of RLIP76. Varying concentrations of either inhibitor were used in an MTT assay. Maximum inhibition was observed near 40 μg/mL by MTT assay (data not shown). Anti-RLIP76 demonstrated greater cell kill in malignant cell lines (p<0.01); pre-immune IgG caused no significant cell kill. Maximal susceptibility was observed with the melanoma cell lines and the SCLC cell line (FIG. 2). Cell density measurements were performed using a hemocytometer to count dye-excluding cells resistant to staining with trypan blue. Approximately 2×10⁴ cells were plated into each well of 96-well flat-bottomed microtiter plate 24 hours prior to addition of medium containing varying concentrations of either pre-immune serum or anti-RLIP76 IgG (0-100 μg/mL final concentration). MTT assays were carried out 96 hours later using methods known in the art (see, e.g., Awasthi, et al., Int J Cancer 1996; 68:333-9) with eight replicate wells per measurement and three separate experiments to determine IC₅₀. Measured absorbance values were directly linked with a spreadsheet for calculation of IC₅₀, defined as the concentration that reduced formazan formation by 50%.

[0033] si-RNA⁵⁰⁸⁻⁵²⁸ was targeted to region of RLIP76 and corresponds closely with a region spanning amino acids 171-185 (nucleotide 510-555 starting from 1 AUG codon in the open reading frame) in the N-terminal region of RLIP76 si-RNA⁵⁰⁸⁻⁵²⁸ is particularly novel in that it lacks homology with other proteins or nucleotide sequences. More details regarding its preparation and described later.

[0034] With RLIP76 si-RNA⁵⁰⁸⁻⁵²⁸ (at a concentration of 20 μg/ml), there was a complete depletion of RLIP76 protein after 24 to 48 hours. Non-malignant cells were less sensitive to RLIP76 depletion as compared with malignant cells. For example, 10 μg/ml si-RNA affected B16 melanoma cells significantly more than HLMVEC (FIG. 3). The time for maximal depletion of RLIP76 protein was also determined by exposing cells to 40 μg/ml RLIP76 si-RNA⁵⁰⁸⁻⁵²⁸ and performing Western-blot analyses after varying time of expo-

sure. The relative resistance of non-malignant cells to RLIP76 depletion by si-RNA is shown in FIG. 4, where several non-malignant cell lines (HLMVEC, HLBE, HAVSM, and HUVEC) were less affected with respect to RLIP76 protein at 24 and 48 h as compared with the malignant cell lines (B16, DG1, Ovar-3, PC-3, H358, and H1618). MTT cytotoxicity assay, RLIP76 si-RNA⁵⁰⁸⁻⁵²⁸ killed the malignant cells in a concentration dependent manner with relative sparing of the non-malignant cells (FIG. 5). The relative efficacy of cell kill was greater with the si-RNA (FIG. 5; FIG. 2D) as compared with anti-RLIP76 IgG (FIG. 4; FIG. 2A). TUNEL assay for apoptosis was performed with anti-RLIP76 IgG showing data consistent with those observed for the MTT assay in which with greater apoptosis was found in malignant cells (data not shown).

[0035] TUNEL assays were measured on adherent cells and floating cells. For adherent cells, approximately 1×10^6 cells were placed into 12-well plates containing coverslips and allowed to incubate with either pre-immune serum or anti-RLIP76 IgG (37 μ g/ml final concentration) for about 24 hours. For floating cells, approximately 1×10^6 cells were grown for 24 hours in 12-well plates then incubated with either pre-immune serum or anti-RLIP76 IgG (37 μ g/ml final concentration) before transferring to histological slides. Apoptosis was then determined by labeling DNA fragmentations using a terminal deoxynucleotidyl-transferase dUTP end labeling method as known in the art. Free 3'-OH DNA ends (characteristic of DNA fragmentation) were labeled using a reaction catalyzed by terminal deoxynucleotidyl-transferase (TdT). Samples were analyzed by laser scanning fluorescence microscope. Apoptotic cells showed green fluorescence and characteristic cell shrinkage.

[0036] Anti-RLIP76, si-RNA or antisense DNA caused complete regression of B16 melanoma in mice. C57B mice were injected on their flanks with 1×10^6 B16-F1 melanoma cells and tumors were measured by calipers daily. When the surface area of the tumor (product of bi-dimensional measurements) exceeded 40 mm² (day 11), animals were injected intraperitoneally with 100 μ l diluent alone (phosphate buffered saline, PBS) or the same volume of diluent containing 200 μ g anti-RLIP76 IgG, RLIP76 si-RNA⁵⁰⁸⁻⁵²⁸, or RLIP76 phosphorothioate antisense⁵⁰⁸⁻⁵²⁸. Additional control animals were injected with pre-immune IgG, scrambled si-RNA or antisense si-RNA. Tumors regressed completely in all animals treated with any inhibitor of RLIP76, whereas uncontrolled growth was observed in the control groups (FIG. 6A). FIG. 6B-6J show representative images of animals in all treatment groups taken eight days after treatment. Animal data demonstrates the dramatic in vivo effect an anti-RLIP76 agent has on tumor regression.

[0037] The region spanning amino acid residues 171-185 (nucleotide 510-555 starting from 1 AUG codon in the open reading frame) in the N-terminal region of RLIP76 was chosen as the target region for synthesis of phosphorothioate DNA. The oxygen in the backbone of the DNA molecules was replaced by sulfur in each phosphate group, which makes the DNA backbone resistant to nucleases. However, the macromolecule remains electrically charged, impeding its passage across cell membrane. Selected DNA sequence was subjected to blast-search (NCBI database) against EST libraries, to ensure that only the selected gene was targeted. Chemically synthesized phosphorothioate DNA in desalted form was purchased from Biosynthesis Inc. A 21-nucleotide long scrambled phosphorothioate DNA was used as a control. The

scrambled DNA sequence was not homologous with RLIP76 cDNA in a blast-search against RLIP76. The targeted cDNA sequence (SEQ ID NO: 19 or AAGAAAAAGCCAATTCAGGAGCC) corresponded to nucleotides 508-528. A corresponding phosphorothioated DNA sequence was GGCTCCTGAATTGGCTTTTTC (SEQ ID NO: 20). The sequence of the scrambled DNA was CATCGAAATCGTTGCAGTTAC (SEQ ID NO: 21). Transfection of phosphorothioate DNA was performed using a transfection kit known in the art; cells were assayed for silencing 24 hours after transfection.

[0038] C57 BL/6 mice were obtained and colonies bred in accordance with standard institutional protocols. In brief, twenty-one 16-week-old mice were divided into seven groups of 3 animals (PBS control; pre-immune IgG control; scrambled si-RNA control; scrambled phosphorothioate oligonucleotide control; anti-RLIP76 IgG, RLIP76⁵⁰⁸⁻⁵²⁸ si-RNA, and RLIP_b 76⁵⁰⁸⁻⁵²⁸ phosphorothioate antisense oligonucleotide). Each of 21 animals was injected with 2×10^6 B16 mouse melanoma cell suspensions in 100 μ l of PBS, subcutaneously. Animals were examined daily for signs of tumor growth. Treatment was administered when the tumor surface area exceeded 45 mm² (at day 11). Treatment consisted of 200 μ g in 100 μ l PBS of either anti-RLIP76 IgG, RLIP76⁵⁰⁸⁻⁵²⁸ si-RNA, or RLIP76⁵⁰⁸⁻⁵²⁸ phosphorothioate antisense oligonucleotide. Control groups were 200 μ g/100 μ l of either pre-immune IgG, scrambled si-RNA, or scrambled phosphorothioate antisense, or diluent (PBS) alone. Tumors were measured in two dimensions using calipers on days 1, 3, 5, 7, 9, 11, 13, 15, 17 and 20.

[0039] The present invention demonstrates the over-expression of RLIP76 in most malignant cells that undergo uncontrolled cells growth as compared with non-malignant cells that do not undergo uncontrolled cell growth. With greater RLIP76 expression, there was increased transport activity of RLIP76 (as demonstrated using transport agents such as DOX or DNP-SG). With greater protein expression there was also an increased dependence on RLIP76 protein as a transport molecule. The present invention also provides for an in vivo effect of targeted anti-RLIP agents in cells undergoing uncontrolled cell growth. Regression of an established melanoma nodule upon administration of a single dose of an anti-RLIP76 agent only 11 days after tumor implantation was observed in mice. Such results are unlike any observed with other anti-cancer therapies. In culture, si-RNA administration was superior to that observed with anti-RLIP76 IgG; in vivo, treatments appeared to provide similar efficacy when equivalent doses were administered. RLIP76 antibody effects may relate to contributions of antibody-dependent cellular cytotoxicity effects.

[0040] Accordingly, the present invention indicates that increased RLIP76 expression is associated with clinical cancerous growth (uncontrolled cells growth). As such, inhibition of RLIP76 offers a targeted solution to eradicate tumorigenic or malignant cells. RLIP76 appears to be a significant transporter in such cells and the activity of RLIP76 appears to be directly involved in uncontrolled cells growth, malignancy and tumorigenicity.

[0041] To specifically target unwanted tumorigenic or malignant cells, the present invention provides for compositions that antagonize, inhibit or deplete RLIP76 (e.g., inhibit RLIP76 transport activity); such compositions being new and important antineoplastic agents to be used alone or in combination with existing therapies. The antagonists include compounds that interact with a cell-surface domain of

RLIP76 that directly affects RLIP76 transport activity. Suitable compositions include those molecules that inhibit, reduce or deplete transport activity of RLIP76 and may be identified as described below.

[0042] The inventors have recently reported several surface epitope regions of RLIP76 when membrane bound to cells. The surface epitope region was found necessary for optimal transport activity of RLIP76 as described by Yadav et al., *Biochemistry* 2004; 43:16243-53, herein incorporated by reference. One surface epitope region comprises on or about amino acids (aa) 154 to 219 (SEQ ID NO:3 and conservatively modified variants, thereof, including deletions of 1 to 5 residues at the C-terminus and or N-terminus). Another surface epitope region comprises on or about amino acids 171 to 185, corresponding to an aa sequence KPIQEPEVPQIDVPN (SEQ ID NO:4 and conservatively modified variants, thereof, including deletions of 1 to 5 residues at the C-terminus and or N-terminus). Such surface epitope regions are not only necessary for optimal transport activity, they are also useful portions of the protein for the identification of inhibitors of RLIP76 transport activity. For example, a deletion mutant protein lacking amino acids 171 to 185 resulted in loss of hydrophobicity of the protein, decreased association of the protein with artificial liposomes, and decreased transport activity. In addition, cells transfected with 171-185 si-RNA (SEQ ID NO:5) resulted in loss of cell surface expression (e.g., decreased membrane association).

[0043] Accordingly, the present invention identifies regions of the protein acting as surface epitopes and capable of providing antagonists (e.g., inhibitors) for RLIP76. Antagonists, as identified herein, include antibodies directed against one or more surface epitope region (e.g., humanized monoclonal antibody), si-RNA sequences directed against one or more surface epitope regions, as well as small molecules found using chemical library screenings against peptides containing one or more surface epitope regions.

[0044] In one form, surface epitope regions and their variants, as identified herein, are synthesized and immobilized on an inert support material and used to screen chemical libraries for compounds that bind this peptide. Suitable methods for chemical library screening are known to one of ordinary skill in the art. The compounds identified by the screening process are tested in a secondary screen that included a liposomal transport assay to determine efficiency of inhibition of RLIP76. RLIP76 inhibitors are also tested in animals alone and in combination with existing anti-seizure medicines in order to evaluate safety and efficacy of each identified inhibitor.

[0045] SEQ ID NO:3 and SEQ ID NO:4 were identified from a series of deletion mutant proteins to RLIP76 (data not shown; see Yadav et al., 2004). In brief, a series of deletion mutants were prepared by PCR-based site-directed mutagenesis using a clone of the full length RLIP76 in an expression vector [pET30a(+)] as template and upstream primer 5' GCG-GATCCATGACTGAGTGCTTCCT (SEQ ID NO:5: BamHI restriction site is underlined) and downstream primer 5'CCGCTCGAGTAGATGGACGTCTCCTTCCTATCCC (SEQ ID NO:6; XhoI restriction site underlined). Mutants included those having deletions of amino acids 203 to 219 (del 203-219), 154 to 171 (del 154-171), 171 to 185 (del 171-185), 154 to 219 (del 154-219), 415 to 448 (del 415-448) and 65 to 80 (del 65-80). The mutagenic primers for del 203-219: 5' GTAGAGAGGACCATGGTAGAGAAG-TATGGC 3' (SEQ ID NO: 7) with its reverse complement);

for del 154-171: 5' AAGAAGTCAAAAGACAAGCCAAT-TCAGGAG (SEQ ID NO: 8; with its reverse complement); for del 171-185: 5' GAAGAAAAAGAACTCAAAC-CCATTTT 3' (SEQ ID NO: 9; with its reverse complement); for del 154-219: 5' GAAGAAGTCAAAAGACGTA-GAGAAGTATGGC 3' (SEQ ID NO: 10; with its reverse complement); for del 415-448: 5' GAATTGTTTACATCGA-CAGGAGTGTGAAACC (SEQ ID NO: 11; with its reverse complement); and for del 65-80: 5' GTGTCTGATGATAG-GACTGAAGGCTATG 3' (SEQ ID NO: 12 and its reverse complement).

[0046] [00461 The template and each deletion mutant was expressed in *E. coli* and after bacterial lysis (with e.g., 1% (w/v) C₁₂E₉ in lysis buffer), the protein was extracted by methods known to one of ordinary skill in the art. For the full length protein and the deletion mutants, one method of protein purification to nearly homogeneity from bacterial extracts used DNP-SG affinity resins (for full description see in Awasthi, et al. *Biochemistry* 2000: 39:9327-9334, herein incorporated by reference). Introduction of deletions specified above in wild type RLIP76 did not affect the affinity of protein with DNP-SG; all deletion mutants could be purified by DNP-SG affinity chromatography. Protein purity was ascertained by SDS-PAGE, Western blot and amino acid composition analysis using methods known in the art and as described in Awasthi et al. 2000. The authenticity of the mutation and the absence of other fortuitous mutations were confirmed by DNA sequencing for each of the deletion mutants.

[0047] Full-length RLIP76 (wt-RLIP76) and deletion mutants (del 203-219, del 154-171, del 171-185, del 154-219, del 65-80, del 415-448 and del 65-80) were expressed as recombinant (rec) proteins in *E. coli* (using pET30a(+)) plasmid under the control of the lac UV5 promoter. Single bacterial colonies were used to induce protein expression. To facilitate extraction of the rec-RLIP76 and its various deletion mutants, bacterial lysates were collected, sonicated, and incubated. After incubation, each reaction mixture was centrifuged and the supernatant fraction was obtained as a cytosol fraction and the pellet was the membrane fraction. The membrane fraction was resuspended in 1% polidocanol (a non-ionic detergent) sonicated again, incubated and collected in the supernatant after centrifugation.

[0048] When extracted in detergent-containing buffer, the ratio of RLIP76 in the detergent/aqueous extracts was found to be 2.5 for the wild-type protein, but decreased to 0.7 in the mutant in which aa 154-219 (SEQ ID NO:4) were deleted (data not shown; see Yadav et al., 2004). Deletion of only one segment of this region (del 171-185 or SEQ ID NO:3) alone resulted in a significant decrease in this ratio to 1.0. For the mutants with deletions within the region from aa 154-219, loss of hydrophobicity correlated with decreased incorporation of mutants into artificial liposomes, and decreased transport activity. The data indicates that the 154-219 region of RLIP76 significantly affects protein partitioning between cytosol and membranes; Residues 171-185 contribute significantly to this effect.

[0049] Functional reconstitution of purified RLIP76 from *E. coli* for transport studies was performed using methods known to one of ordinary skill in the art, an example of which is described in Awasthi, et al. 2000. The degree of incorporation of wild-type as well as mutant RLIP76 into artificial liposomes was assessed by measuring RLIP76 after centrifugation (pellet and supernatant of prepared liposomes) by

ELISA assay using anti-RLIP76 antibodies. Measurement of the transport of a cationic agent, doxorubicin (DOX), in the reconstituted liposomes was performed using methods known to one of ordinary skill in the art, an example of which is described in Awasthi, et al. 2000. The ATP-dependent uptake of [C^{14}]-DOX (specific activity 8.4×10^4 cpm/nmol) was determined by subtracting the radioactivity (cpm) of the control without ATP from that of the experimental containing ATP. Transport of DOX was calculated in terms of pmol/min/mg protein. The transport of [3H]-DNP-SG (specific activity 3.2×10^3 cpm/nmol) was measured using methods known to one of ordinary skill in the art, an example of which is described in Awasthi, et al. 2000.

[0050] The majority (87%) of total wild-type RLIP76 was found in the pellet fraction, incorporated into the proteoliposomes. Deletion of aa 203-219 or 154-171 decreased incorporation slightly (to 83 and 80%, respectively). Deletion of aa 171-185 significantly effected incorporation of the protein into proteoliposomes (64%) as did deletion of residues 154-b219, with only 33% of total protein found incorporated into proteoliposomes. Deletions affecting the ATP-binding sites (aa 65-80 and aa 415-448) had no significant effect on the amount of protein incorporated into proteoliposomes. Thus region 154-219 is an important determinant of membrane insertion.

[0051] For ATP-dependent transport of molecules across the proteoliposomes, transport was significantly decreased (21%) in the mutant lacking aa 154-171 (27.6 versus 21.7 nmol/min/mg for transport of DOX by the full length RLIP76 versus the deletion mutant, $p < 0.05$). Deletion of aa 171-185 resulted in approximately 40% loss of transport activity for DOX and a similar loss (35%) in transport activity for dinitrophenyl S-glutathione (DNP-SG). Deletion of the entire 154-219 region resulted in further significant loss (50%) of transport activity for both DOX and DNP-SG. Because deletion of ATP-binding site regions did not affect partitioning of the mutants between cytosol and membrane, the observed decrease in transport activity of deletion mutant aa 154-219 is believed due to loss of protein association with the membrane because of its decreased partitioning in the membrane.

[0052] The effect in eukaryotes of losing surface epitope regions spanning residues 171-185 (SEQ ID NO:4) or 154-219 (SEQ ID NO:3) was similar to that described above (data not shown; see Yadav et al., 2004). When H358 cells were transfected with an empty vector (pcDNA3.1) or a vector containing either full length RLIP76 or its deletion mutants lacking aa 171-185 or aa 154-219, membrane association of RLIP76 was significantly reduced in cells transfected with the deletion mutants, as analyzed by Western blots. Hence, the aa 154-219 region is a determinant of the membrane association of RLIP76 and it is independent of whether the protein is expressed in eukaryotes or prokaryotes. Immunohistochemistry studies using anti-RLIP76 antibodies raised against full-length RLIP76 were performed with live, unfixed H358 wild-type cells and examined by confocal laser microscopy and showed a staining pattern consistent with cell-surface localization. RLIP76 co-localized with another protein, her2/neu, known to have a cell-surface domain. Anti-RLIP76 antibody was detected using a rhodamine red-x-conjugated secondary antibody, and anti-her2/neu antibody using an FITC tagged secondary antibody. Cell-surface epitopes were recognized by both anti-RLIP76 and her2/neu antibodies which co-localized in unfixed cells indicating that RLIP76 had cell-surface epitopes just like her2/neu.

[0053] H358 cells constitutively express a wild-type RLIP76. The wild-type was removed by treating H358 cells with si-RNA directed at the region encoding aa 171-185, to silence the expression of wild-type RLIP76, while leaving the expression of 171-185 mutant unaffected. For this, a 23-nucleotide sequence motif comprising AA(N19)TT or NA(N21) (N, any nucleotide) with approximately 50% GC content was searched for. The sequence of sense si-RNA corresponds to N21. The 3' end of the sense si-RNA was converted to TT to generate a symmetric duplex with respect to the sequence composition of sense and antisense 3' overhangs. The selected si-RNA sequence was subjected to blast-search (NCBI database) against EST libraries, to ensure that only one gene was targeted. Chemically synthesized si-RNA duplex in the 2' de-protected and desalted forms, was purchased from Dharmacon. A 23-nucleotide long scrambled si-RNA duplex was used as a control. The scrambled si-RNA sequence was not homologous with RLIP76 mRNA in a blast-search against RLIP76. The targeted cDNA sequence was AAGAAAAAGCCAATTCAGGAGCC (SEQ ID NO:13) corresponding to nucleotides 508 to 528. The corresponding sense si-RNA sequence was GAAAAAGCCAAUUCAGGAGCCdTdT (SEQ ID NO: 14) and the antisense si-RNA sequence was GGCUCCUGAAUUGGCUUUUUCdTdT (SEQ ID NO: 15). The sequences of the scrambled si-RNA in the sense and antisense directions were GUACUGCAACGAUUUCGAUGdTdT (SEQ ID NO: 16) and CAUCGAAAUCGUUGCAGUUCdTdT (SEQ ID NO: 17), respectively.

[0054] Transfection of si-RNA duplexes was performed using a kit (Transmessenger Transfection Reagent Kit from Qiagen) and assayed for expression about 24 hours later. Cells (approximately 3×10^6) were placed into six-well plates and after about 24 hours were incubated for about 3 hours with RLIP76 si-RNA or scrambled si-RNA in an appropriate transfection reagent. Excess si-RNA was washed off with PBS and medium was added. Cell samples were pelleted, solubilized in a lysis buffer (10 mM Tris-HCl, pH 7.4, containing 1.4 mM β -mercaptoethanol, 100 μ M EDTA, 50 μ M BHT, 100 μ M PMSF and 1% polidocanol), sonicated and then incubated for about 4 h in the cold ($4^\circ C$). Afterwards, each sample was centrifuged and supernatants (containing both cytosolic proteins and solubilized membrane proteins) collected and analyzed by Western blot analyses according to a method provided by Towbin et al. (Towbin, et al. PNAS 1979; 76:4350-4353) using anti-RLIP76 IgG as well as IgG against the peptide 171-185. Gel bands were quantified by scanning densitometry. Polyclonal antibodies against various deleted epitope regions of RLIP76 were custom made. The peptide antibodies as well as pre-immune serum were purified by DE-52 anion exchange chromatography, followed by protein-A-Sepharose affinity chromatography to obtain pure IgG fractions. Immuno-reactivity and specificity of these peptides using their respective purified IgG were checked by dot blot analyses.

[0055] The si-RNA 171-185 effectively silenced wild-type RLIP76 expression in the untransfected, empty-vector-transfected, as well as wild-type RLIP76 transfected cells (data not shown; see Yadav et al., 2004). Antibodies against the 171-185 peptide failed to detect RLIP76 antigen, while antibodies against full-length RLIP76 recognized the persistent presence of the residual deletion mutant RLIP76. Western blotting against the anti-del 171-185 antibody showed no signal in the RLIP76 deletion mutant transfected cells confirming that

expression of wild-type RLIP76 was effectively blocked in these cells. Cell surface expression of RLIP76 in del 171-185 transfected cells with or without pre-treatment with si-RNA directed at aa 171-185 using immunohistochemical analysis and an anti-del 171-185 antibody showed that cells with control si-RNA had significant cell surface signal which was absent in cells in which RLIP had been silenced by the si-RNA. RLIP76 is, thus, an integral membrane protein with at least one cell surface domain spanning amino acids 154 to 219.

[0056] Accordingly, the present invention provides several surface epitope regions of RLIP that, when altered, blocked or deleted, prevent RLIP from performing its transport function. Compositions of the present invention include the several surface epitope regions as well as use of these surface epitope regions to obtain specific inhibitors of RLIP that are capable of altering, inhibiting or the transport function of RLIP. The inhibitors include si-RNAs, each having a sequence directed against the one or more surface epitope regions as well as phosphorothioate antisense oligonucleotides directed against such surface epitope regions (e.g., GGCTCCTGAATTG-GCTTTTTC; SEQ ID NO:18) and a corresponding silencing

RNA sequence to the phosphorothioate antisense oligonucleotides (e.g., AAGAAAAGCCAATTCAGGAGCC; SEQ ID NO: 19), where, SEQ ID NO: 19 corresponds with the targeted cDNA sequence for nucleotides 508 to 528 used to generate the antisense si-RNA or SEQ ID NO: 13. In addition, inhibitors include antibodies (monoclonal and/or polyclonal) directed against the one or more surface epitope regions, such regions including SEQ ID NO: 3 and SEQ ID NO:4. Moreover, the inhibitors identified herein provide compounds for anti-seizure medicines. Importantly, the inhibitors are additional targets for identifying important compounds and small molecule from chemical library screenings, wherein the identified compounds and/or small molecules are effective as anti-cancer medicines.

[0057] While particular embodiments of the invention and method steps of the invention have been described herein, additional alternatives not specifically disclosed but known in the art are intended to fall within the scope of the invention. Thus, it is understood that other embodiments and applications of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the appended claims and drawings.

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c a g a a a t c t t	t t a a a c g a c t	g a c c t t c c t t	a a a a c a g a a	t g a c t c c g a t	t g c t t g c t t g	3043
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t t g a a a t t a t	c c a t a t c a t c	t c a g t g a a c c	t a c t g g t g g a	c t c c c a a t t g	a c a a g a t t g a	3163
g c a a t a g a a a	a a a a t t c c t t	t c c t t t g a a t	g a t a g c t g t g	a t t c a c c c c a	c c c c a t t t t c	3223
t t g t t t c t g g	t c c a t c c g a t	g a g a c g g a t g	c t c t g a t g c t	c t g a g g c t t c	t g g g a g g c t g	3283
g g c c c t g g a g	g c a a c g t g c t	g c a g g c g c a c	t e t g t c a g a g	t g a a c a g c a c	c g c g a g a c a g	3343
g c c a g g c t c g	t g g c t c g g a a	g a c a a a c c c c	a c a c a c a c t c	a a g g g g t c g a	a a a c a a a c c c	3403
c a c a c g a g g g	c t c t c a c c t c	c t t c t c c t a g	g t a g t a t t t a	t t t t c a g c a c	c t g t t t g a t g	3463
c a g t t t t t a a	t c c t c t a c c t	a t t g c a c t g t	t g t g a c t c g t	t g g c c a t t a t	t t g a t t t t t g	3523
t a c g a a a a a a	a g c t t t g t t a	t a g a a a t c a g	c a t a c t a t t t	t t t t a a a t c t	g g a g a g a a g a	3583
t a t t c t g g t g	a c t g a a a g t a	t g g t c g g g t g	t c a g a t a t a a	a t g t g c a a a t	g c c t t c t t g c	3643
t g t c c t g t c g	g t c t c a g t a c	g t t c a c t t t a	t a g c t g c t g g	c a a t a t c g a a	g g t t c c t t t t	3703
t t g t t t g t g t	a a a c t c t a a t	t t c t a t c a a g	g t g t c a t g g a	t t t t t a a a a t	t a g t a t t t c a	3763
t t a c a a a t g t	c t c a g c a t t g	g t t a a c t a a t	t t t t g c c a g g	a c c a t t a t t g	a t c a a g c a a a	3823
t a a a t t c a a c	a g c c a t t t g g	g a a a a g a a a	a g c t t c t a g t	t t t t t t g t a c	a c a t t c t t t c	3883
t g t g a g g a g a	t t g a g t a c t c	t g c a g c t g g c	g a g g a g t t g g	t t g a g g c a c t	t c t t c a a g g c	3943
c a a g g g g g a a	c a c a g t g t t t	t g t t t c a g c	t c a c t t t g t a	c c c c t c a c c t	c t g c a g a c a c	4003
g g g g a g a a c c	c c g g a c c c c t	g g c a t g c a t g	c t g g c g g c g g	c a t g c c t c c c	t t c c a c a a g c	4063
c c a t g c t g c t	g c a g a g g g a g	c c t g t g t t g	c a a a a c c c a g	t g g a c t g g g c	t g g g t c t g c t	4123
g t c t g a g c a g	c t c c t g g c t c	c g g t g g g a a c	t g c a c a c a a g	t c c a c t g g c c	t g g c t t g g c c	4183
c c a g g c a t t g	c a a t t g a c a g	a c a t t t g c a t	t t c a t a c g g t	a a a t g a g g a c	t c a g c a c a g c	4243
c a a c c a t a a t	c a g c a t g t c t	g g g a t a g a c t	g g t c t a g a a t	a a a a a t g a a g	t t t c c a t t g c	4303
t t t g t t t g c t	t t a a a a a t t c	c a c a a t t a a a	a t a t c t g t c a	t t g a a a g c t t	a a a a a a a a a a	4363
a a a a a						4368

<210> SEQ ID NO 2

<211> LENGTH: 655

<212> TYPE: PRT

<213> ORGANISM: homo sapiens

<400> SEQUENCE: 2

Met Thr Glu Cys Phe Leu Pro Pro Thr Ser Ser Pro Ser Glu His Arg
 1 5 10 15

Arg Val Glu His Gly Ser Gly Leu Thr Arg Thr Pro Ser Ser Glu Glu
 20 25 30

Ile Ser Pro Thr Lys Phe Pro Gly Leu Tyr Arg Thr Gly Glu Pro Ser
 35 40 45

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Pro	Pro	His	Asp	Ile	Leu	His	Glu	Pro	Pro	Asp	Val	Val	Ser	Asp	Asp
50						55					60				
Glu	Lys	Asp	His	Gly	Lys	Lys	Lys	Gly	Lys	Phe	Lys	Lys	Lys	Glu	Lys
65					70					75					80
Arg	Thr	Glu	Gly	Tyr	Ala	Ala	Phe	Gln	Glu	Asp	Ser	Ser	Gly	Asp	Glu
				85					90					95	
Ala	Glu	Ser	Pro	Ser	Lys	Met	Lys	Arg	Ser	Lys	Gly	Ile	His	Val	Phe
			100					105					110		
Lys	Lys	Pro	Ser	Phe	Ser	Lys	Lys	Lys	Glu	Lys	Asp	Phe	Lys	Ile	Lys
		115					120					125			
Glu	Lys	Pro	Lys	Glu	Glu	Lys	His	Lys	Glu	Glu	Lys	His	Lys	Glu	Glu
		130				135					140				
Lys	His	Lys	Glu	Lys	Lys	Ser	Lys	Asp	Leu	Thr	Ala	Ala	Asp	Val	Val
145					150				155						160
Lys	Gln	Trp	Lys	Glu	Lys	Lys	Lys	Lys	Lys	Lys	Pro	Ile	Gln	Glu	Pro
				165					170					175	
Glu	Val	Pro	Gln	Ile	Asp	Val	Pro	Asn	Leu	Lys	Pro	Ile	Phe	Gly	Ile
			180					185						190	
Pro	Leu	Ala	Asp	Ala	Val	Glu	Arg	Thr	Met	Met	Tyr	Asp	Gly	Ile	Arg
		195					200					205			
Leu	Pro	Ala	Val	Phe	Arg	Glu	Cys	Ile	Asp	Tyr	Val	Glu	Lys	Tyr	Gly
		210				215					220				
Met	Lys	Cys	Glu	Gly	Ile	Tyr	Arg	Val	Ser	Gly	Ile	Lys	Ser	Lys	Val
225					230					235					240
Asp	Glu	Leu	Lys	Ala	Ala	Tyr	Asp	Arg	Glu	Glu	Ser	Thr	Asn	Leu	Glu
				245					250					255	
Asp	Tyr	Glu	Pro	Asn	Thr	Val	Ala	Ser	Leu	Leu	Lys	Gln	Tyr	Leu	Arg
			260					265					270		
Asp	Leu	Pro	Glu	Asn	Leu	Leu	Thr	Lys	Glu	Leu	Met	Pro	Arg	Phe	Glu
		275					280					285			
Glu	Ala	Cys	Gly	Arg	Thr	Thr	Glu	Thr	Glu	Lys	Val	Gln	Glu	Phe	Gln
		290				295					300				
Arg	Leu	Leu	Lys	Glu	Leu	Pro	Glu	Cys	Asn	Tyr	Leu	Leu	Ile	Ser	Trp
305					310				315						320
Leu	Ile	Val	His	Met	Asp	His	Val	Ile	Ala	Lys	Glu	Leu	Glu	Thr	Lys
				325					330					335	
Met	Asn	Ile	Gln	Asn	Ile	Ser	Ile	Val	Leu	Ser	Pro	Thr	Val	Gln	Ile
			340					345					350		
Ser	Asn	Arg	Val	Leu	Tyr	Val	Phe	Phe	Thr	His	Val	Gln	Glu	Leu	Phe
		355					360					365			
Gly	Asn	Val	Val	Leu	Lys	Gln	Val	Met	Lys	Pro	Leu	Arg	Trp	Ser	Asn
		370				375					380				
Met	Ala	Thr	Met	Pro	Thr	Leu	Pro	Glu	Thr	Gln	Ala	Gly	Ile	Lys	Glu
385					390					395					400
Glu	Ile	Arg	Arg	Gln	Glu	Phe	Leu	Leu	Asn	Cys	Leu	His	Arg	Asp	Leu
				405					410					415	
Gln	Gly	Gly	Ile	Lys	Asp	Leu	Ser	Lys	Glu	Glu	Arg	Leu	Trp	Glu	Val
			420					425					430		
Gln	Arg	Ile	Leu	Thr	Ala	Leu	Lys	Arg	Lys	Leu	Arg	Glu	Ala	Lys	Arg
		435					440					445			
Gln	Glu	Cys	Glu	Thr	Lys	Ile	Ala	Gln	Glu	Ile	Ala	Ser	Leu	Ser	Lys
		450				455					460				

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Glu Asp Val Ser Lys Glu Glu Met Asn Glu Asn Glu Glu Val Ile Asn
 465 470 475 480
 Ile Leu Leu Ala Gln Glu Asn Glu Ile Leu Thr Glu Gln Glu Glu Leu
 485 490 495
 Leu Ala Met Glu Gln Phe Leu Arg Arg Gln Ile Ala Ser Glu Lys Glu
 500 505 510
 Glu Ile Glu Arg Leu Arg Ala Glu Ile Ala Glu Ile Gln Ser Arg Gln
 515 520 525
 Gln His Gly Arg Ser Glu Thr Glu Glu Tyr Ser Ser Glu Ser Glu Ser
 530 535 540
 Glu Ser Glu Asp Glu Glu Glu Leu Gln Ile Ile Leu Glu Asp Leu Gln
 545 550 555 560
 Arg Gln Asn Glu Glu Leu Glu Ile Lys Asn Asn His Leu Asn Gln Ala
 565 570 575
 Ile His Glu Glu Arg Glu Ala Ile Ile Glu Leu Arg Val Gln Leu Arg
 580 585 590
 Leu Leu Gln Met Gln Arg Ala Lys Ala Glu Gln Gln Ala Gln Glu Asp
 595 600 605
 Glu Glu Pro Glu Trp Arg Gly Gly Ala Val Gln Pro Pro Arg Asp Gly
 610 615 620
 Val Leu Glu Pro Lys Ala Ala Lys Glu Gln Pro Lys Ala Gly Lys Glu
 625 630 635 640
 Pro Ala Lys Pro Ser Pro Ser Arg Asp Arg Lys Glu Thr Ser Ile
 645 650 655

<210> SEQ ID NO 3
 <211> LENGTH: 66
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: epitope region comprising residues 154-219

<400> SEQUENCE: 3

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

<210> SEQ ID NO 4
 <211> LENGTH: 15
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: epitope region comprising residues 171-185

<400> SEQUENCE: 4

Lys Pro Ile Gln Glu Pro Glu Val Pro Gln Ile Asp Val Pro Asn
 1 5 10 15

<210> SEQ ID NO 5

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<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: upstream primer sequence for clone of full length RLIP

<400> SEQUENCE: 5
ggcggatcca tgactgagtg cttcct 26

<210> SEQ ID NO 6
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: downstream primer sequence for clone of full length RLIP

<400> SEQUENCE: 6
ccgctcgagt agatggacgt ctccttccta tccc 34

<210> SEQ ID NO 7
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutagenic upstream primer for deletion mutant 203-219

<400> SEQUENCE: 7
gtagagagga ccatggtaga gaagtatggc 30

<210> SEQ ID NO 8
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutagenic upstream primer for deletion mutant 154-171

<400> SEQUENCE: 8
gaagaagtca aaagacaagc caattcagga g 31

<210> SEQ ID NO 9
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutagenic upstream primer for deletion mutant 171-185

<400> SEQUENCE: 9
gaagaaaaag aaactcaaac ccattttt 28

<210> SEQ ID NO 10
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutagenic upstream prime for deletion mutant 154-219

<400> SEQUENCE: 10
gaagaagtca aaagacgtag agaagtatgg c 31

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<210> SEQ ID NO 11
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutagenic upstream primer for deletion mutant
415-448

<400> SEQUENCE: 11

gaattgttta catcgacagg agtgtgaaac c 31

<210> SEQ ID NO 12
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: mutagenic upstream primer for deletion mutant
65-80

<400> SEQUENCE: 12

gtgtctgatg ataggactga aggctatg 28

<210> SEQ ID NO 13
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: targeted cDNA corresponding to nucleotides
508-528 of ral A binding protein 1

<400> SEQUENCE: 13

aagaaaaagc caattcagga gcc 23

<210> SEQ ID NO 14
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: corresponding sense siRNA strand for targeted
cDNA
<220> FEATURE:
<221> NAME/KEY: d
<222> LOCATION: (22)..(23)
<223> OTHER INFORMATION: dT

<400> SEQUENCE: 14

gaaaaagcca auucaggagc cdd 23

<210> SEQ ID NO 15
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: corresponding antisense siRNA strand for
targeted cDNA
<220> FEATURE:
<221> NAME/KEY: d
<222> LOCATION: (22)..(23)
<223> OTHER INFORMATION: dT

<400> SEQUENCE: 15

ggcuccugaa uuggcuuuuu cdd 23

<210> SEQ ID NO 16
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: scrambled sense siRNA strand
<220> FEATURE:
<221> NAME/KEY: d
<222> LOCATION: (22)..(23)
<223> OTHER INFORMATION: dT

<400> SEQUENCE: 16

guaacugcaa cgauuucgau gdd 23

<210> SEQ ID NO 17
<211> LENGTH: 23
<212> TYPE: RNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: scrambled antisense siRNA strand
<220> FEATURE:
<221> NAME/KEY: d
<222> LOCATION: (22)..(23)
<223> OTHER INFORMATION: dT

<400> SEQUENCE: 17

caucgaaauc guugcaguua cdd 23

<210> SEQ ID NO 18
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: phosphorothioate antisense oligonucleotides

<400> SEQUENCE: 18

ggctcctgaa ttggcttttt c 21

<210> SEQ ID NO 19
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: corresponding silencing RNA sequence to the
phosphorothioate antisense oligonucleotide

<400> SEQUENCE: 19

aagaaaaagcc aattcaggag cc 22

<210> SEQ ID NO 20
<211> LENGTH: 198
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: epitope nucleotide sequence corresponding to
residues 154-219

<400> SEQUENCE: 20

ttgacagcag ctgatgttgt taaacagtgg aaggaaaaga agaaaaagaa aaagccaatt 60
caggagccag aggtgcctca gattgatgtt ccaaatctca aaccattttt tggaattcct 120
ttggctgatg cagtagagag gaccatgatg tatgatggca ttcggctgcc agccgttttc 180
cgtgaatgta tagattac 198

<210> SEQ ID NO 21
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:

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<223> OTHER INFORMATION: epitope nucleotide sequence corresponding to residues 171-185

<400> SEQUENCE: 21

aagccaattc aggagccaga ggtgcctcag attgatgttc caaat 45

<210> SEQ ID NO 22
 <211> LENGTH: 1968
 <212> TYPE: DNA
 <213> ORGANISM: homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1965)

<400> SEQUENCE: 22

atg act gag tgc ttc ctg ccc ccc acc agc agc ccc agt gaa cac cgc 48
 Met Thr Glu Cys Phe Leu Pro Pro Thr Ser Ser Pro Ser Glu His Arg
 1 5 10 15

agg gtg gag cat ggc agc ggg ctt acc cgg acc ccc agc tct gaa gag 96
 Arg Val Glu His Gly Ser Gly Leu Thr Arg Thr Pro Ser Ser Glu Glu
 20 25 30

atc agc cct act aag ttt cct gga ttg tac cgc act ggc gag ccc tca 144
 Ile Ser Pro Thr Lys Phe Pro Gly Leu Tyr Arg Thr Gly Glu Pro Ser
 35 40 45

cct ccc cat gac atc ctc cat gag cct cct gat gta gtg tct gat gat 192
 Pro Pro His Asp Ile Leu His Glu Pro Pro Asp Val Val Ser Asp Asp
 50 55 60

gag aaa gat cat ggg aag aaa aaa ggg aaa ttt aag aaa aag gaa aag 240
 Glu Lys Asp His Gly Lys Lys Lys Gly Lys Phe Lys Lys Lys Glu Lys
 65 70 75 80

agg act gaa ggc tat gca gcc ttt cag gaa gat agc tct gga gat gag 288
 Arg Thr Glu Gly Tyr Ala Ala Phe Arg Ser Ser Ser Gly Asp Glu
 85 90 95

gca gaa agt cct tct aaa atg aag agg tcc aag gga atc cat gtt ttc 336
 Ala Glu Ser Pro Ser Lys Met Lys Arg Ser Lys Gly Ile His Val Phe
 100 105 110

aag aag ccc agc ttt tct aaa aag aag gaa aag gat ttt aaa ata aaa 384
 Lys Lys Pro Ser Phe Ser Lys Lys Lys Glu Lys Asp Phe Lys Ile Lys
 115 120 125

gag aaa ccc aaa gaa gaa aag cat aaa gaa gaa aag cac aaa gaa gaa 432
 Glu Lys Pro Lys Glu Glu Lys His Lys Glu Glu Lys His Lys Glu Glu
 130 135 140

aaa cat aaa gag aag aag tca aaa gac ttg aca gca gct gat gtt gtt 480
 Lys His Lys Glu Lys Lys Ser Lys Asp Leu Thr Ala Ala Asp Val Val
 145 150 155 160

aaa cag tgg aag gaa aag aag aaa aag aaa aag cca att cag gag cca 528
 Lys Gln Trp Lys Glu Lys Lys Lys Lys Lys Lys Pro Ile Gln Glu Pro
 165 170 175

gag gtg cct cag att gat gtt cca aat ctc aaa ccc att ttt gga att 576
 Glu Val Pro Gln Ile Asp Val Pro Asn Leu Lys Pro Ile Phe Gly Ile
 180 185 190

cct ttg gct gat gca gta gag agg acc atg atg tat gat ggc att cgg 624
 Pro Leu Ala Asp Ala Val Glu Arg Thr Met Met Tyr Asp Gly Ile Arg
 195 200 205

ctg cca gcc gtt ttc cgt gaa tgt ata gat tac gta gag aag tat ggc 672
 Leu Pro Ala Val Phe Arg Glu Cys Ile Asp Tyr Val Glu Lys Tyr Gly
 210 215 220

atg aag tgt gaa ggc atc tac aga gta tca gga att aaa tca aag gtg 720
 Met Lys Cys Glu Gly Ile Tyr Arg Val Ser Gly Ile Lys Ser Lys Val
 225 230 235 240

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gat gag cta aaa gca gcc tat gac cgg gag gag tct aca aac ttg gaa	768
Asp Glu Leu Lys Ala Ala Tyr Asp Arg Glu Glu Ser Thr Asn Leu Glu	
245 250 255	
gac tat gag cct aac act gta gcc agt ttg ctg aag cag tat ttg cga	816
Asp Tyr Glu Pro Asn Thr Val Ala Ser Leu Leu Lys Gln Tyr Leu Arg	
260 265 270	
gac ctt cca gag aat ttg ctt acc aaa gag ctt atg ccc aga ttt gaa	864
Asp Leu Pro Glu Asn Leu Leu Thr Lys Glu Leu Met Pro Arg Phe Glu	
275 280 285	
gag gct tgt ggg agg acc acg gag act gag aaa gtg cag gaa ttc cag	912
Glu Ala Cys Gly Arg Thr Thr Glu Thr Glu Lys Val Gln Glu Phe Gln	
290 295 300	
cgt tta ctc aaa gaa ctg cca gaa tgt aac tat ctt ctg att tct tgg	960
Arg Leu Leu Lys Glu Leu Pro Glu Cys Asn Tyr Leu Leu Ile Ser Trp	
305 310 315 320	
ctc att gtg cac atg gac cat gtc att gca aag gaa ctg gaa aca aaa	1008
Leu Ile Val His Met Asp His Val Ile Ala Lys Glu Leu Glu Thr Lys	
325 330 335	
atg aat ata cag aac att tct ata gtg ctc agc cca act gtg cag atc	1056
Met Asn Ile Gln Asn Ile Ser Ile Val Leu Ser Pro Thr Val Gln Ile	
340 345 350	
agc aat cga gtc ctg tat gtg ttt ttc aca cat gtg caa gaa ctc ttt	1104
Ser Asn Arg Val Leu Tyr Val Phe Phe Thr His Val Gln Glu Leu Phe	
355 360 365	
gga aat gtg gta cta aag caa gtg atg aaa cct ctg cga tgg tct aac	1152
Gly Asn Val Val Leu Lys Gln Val Met Lys Pro Leu Arg Trp Ser Asn	
370 375 380	
atg gcc acg atg ccc acg ctg cca gag acc cag gcg ggc atc aag gag	1200
Met Ala Thr Met Pro Thr Leu Pro Glu Thr Gln Ala Gly Ile Lys Glu	
385 390 395 400	
gag atc agg aga cag gag ttt ctt ttg aat tgt tta cat cga gat ctg	1248
Glu Ile Arg Arg Gln Glu Phe Leu Leu Asn Cys Leu His Arg Asp Leu	
405 410 415	
cag ggt ggg ata aag gat ttg tct aaa gaa gaa aga tta tgg gaa gta	1296
Gln Gly Gly Ile Lys Asp Leu Ser Lys Glu Glu Arg Leu Trp Glu Val	
420 425 430	
caa aga att ttg aca gcc ctc aaa aga aaa ctg aga gaa gct aaa aga	1344
Gln Arg Ile Leu Thr Ala Leu Lys Arg Lys Leu Arg Glu Ala Lys Arg	
435 440 445	
cag gag tgt gaa acc aag att gca caa gag ata gcc agt ctt tca aaa	1392
Gln Glu Cys Glu Thr Lys Ile Ala Gln Glu Ile Ala Ser Leu Ser Lys	
450 455 460	
gag gat gtt tcc aaa gaa gag atg aat gaa aat gaa gaa gtt ata aat	1440
Glu Asp Val Ser Lys Glu Glu Met Asn Glu Asn Glu Glu Val Ile Asn	
465 470 475 480	
att ctc ctt gct cag gag aat gag atc ctg act gaa cag gag gag ctc	1488
Ile Leu Leu Ala Gln Glu Asn Glu Ile Leu Thr Glu Gln Glu Glu Leu	
485 490 495	
ctg gcc atg gag cag ttt ctg cgc cgg cag att gcc tca gaa aaa gaa	1536
Leu Ala Met Glu Gln Phe Leu Arg Arg Gln Ile Ala Ser Glu Lys Glu	
500 505 510	
gag att gaa cgc ctc aga gct gag att gct gaa att cag agt cgc cag	1584
Glu Ile Glu Arg Leu Arg Ala Glu Ile Ala Glu Ile Gln Ser Arg Gln	
515 520 525	
cag cac ggc cga agt gag act gag gag tac tcc tcc gag agc gag agc	1632
Gln His Gly Arg Ser Glu Thr Glu Glu Tyr Ser Ser Glu Ser Glu Ser	
530 535 540	

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gag agt gag gat gag gag gag ctg cag atc att ctg gaa gac tta cag	1680
Glu Ser Glu Asp Glu Glu Glu Leu Gln Ile Ile Leu Glu Asp Leu Gln	
545 550 555 560	
aga cag aac gaa gag ctg gaa ata aag aac aat cat ttg aat caa gca	1728
Arg Gln Asn Glu Glu Leu Glu Ile Lys Asn Asn His Leu Asn Gln Ala	
565 570 575	
att cat gag gag cgc gag gcc atc atc gag ctg cgc gtg cag ctg cgg	1776
Ile His Glu Glu Arg Glu Ala Ile Ile Glu Leu Arg Val Gln Leu Arg	
580 585 590	
ctg ctc cag atg cag cga gcc aag gcc gag cag cag gcg cag gag gac	1824
Leu Leu Gln Met Gln Arg Ala Lys Ala Glu Gln Gln Ala Gln Glu Asp	
595 600 605	
gag gag cct gag tgg cgc ggg ggt gcc gtc cag ccg ccc aga gac ggc	1872
Glu Glu Pro Glu Trp Arg Gly Gly Ala Val Gln Pro Pro Arg Asp Gly	
610 615 620	
gtc ctt gag cca aaa gca gct aaa gag cag cca aag gca ggc aag gag	1920
Val Leu Glu Pro Lys Ala Ala Lys Glu Gln Pro Lys Ala Gly Lys Glu	
625 630 635 640	
ccg gca aag cca tcg ccc agc agg gat agg aag gag acg tcc atc tga	1968
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<211> LENGTH: 655

<212> TYPE: PRT

<213> ORGANISM: homo sapiens

<400> SEQUENCE: 23

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35 40 45	
Pro Pro His Asp Ile Leu His Glu Pro Pro Asp Val Val Ser Asp Asp	
50 55 60	
Glu Lys Asp His Gly Lys Lys Lys Gly Lys Phe Lys Lys Lys Glu Lys	
65 70 75 80	
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85 90 95	
Ala Glu Ser Pro Ser Lys Met Lys Arg Ser Lys Gly Ile His Val Phe	
100 105 110	
Lys Lys Pro Ser Phe Ser Lys Lys Lys Glu Lys Asp Phe Lys Ile Lys	
115 120 125	
Glu Lys Pro Lys Glu Glu Lys His Lys Glu Glu Lys His Lys Glu Glu	
130 135 140	
Lys His Lys Glu Lys Lys Ser Lys Asp Leu Thr Ala Ala Asp Val Val	
145 150 155 160	
Lys Gln Trp Lys Glu Lys Lys Lys Lys Lys Pro Ile Gln Glu Pro	
165 170 175	
Glu Val Pro Gln Ile Asp Val Pro Asn Leu Lys Pro Ile Phe Gly Ile	
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Pro Leu Ala Asp Ala Val Glu Arg Thr Met Met Tyr Asp Gly Ile Arg	
195 200 205	
Leu Pro Ala Val Phe Arg Glu Cys Ile Asp Tyr Val Glu Lys Tyr Gly	
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Met	Lys	Cys	Glu	Gly	Ile	Tyr	Arg	Val	Ser	Gly	Ile	Lys	Ser	Lys	Val	225	230	235	240
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Asp	Tyr	Glu	Pro	Asn	Thr	Val	Ala	Ser	Leu	Leu	Lys	Gln	Tyr	Leu	Arg	260	265	270	
Asp	Leu	Pro	Glu	Asn	Leu	Leu	Thr	Lys	Glu	Leu	Met	Pro	Arg	Phe	Glu	275	280	285	
Glu	Ala	Cys	Gly	Arg	Thr	Thr	Glu	Thr	Glu	Lys	Val	Gln	Glu	Phe	Gln	290	295	300	
Arg	Leu	Leu	Lys	Glu	Leu	Pro	Glu	Cys	Asn	Tyr	Leu	Leu	Ile	Ser	Trp	305	310	315	320
Leu	Ile	Val	His	Met	Asp	His	Val	Ile	Ala	Lys	Glu	Leu	Glu	Thr	Lys	325	330	335	
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Ser	Asn	Arg	Val	Leu	Tyr	Val	Phe	Phe	Thr	His	Val	Gln	Glu	Leu	Phe	355	360	365	
Gly	Asn	Val	Val	Leu	Lys	Gln	Val	Met	Lys	Pro	Leu	Arg	Trp	Ser	Asn	370	375	380	
Met	Ala	Thr	Met	Pro	Thr	Leu	Pro	Glu	Thr	Gln	Ala	Gly	Ile	Lys	Glu	385	390	395	400
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Ile	Leu	Leu	Ala	Gln	Glu	Asn	Glu	Ile	Leu	Thr	Glu	Gln	Glu	Glu	Leu	485	490	495	
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Arg	Gln	Asn	Glu	Glu	Leu	Glu	Ile	Lys	Asn	Asn	His	Leu	Asn	Gln	Ala	565	570	575	
Ile	His	Glu	Glu	Arg	Glu	Ala	Ile	Ile	Glu	Leu	Arg	Val	Gln	Leu	Arg	580	585	590	
Leu	Leu	Gln	Met	Gln	Arg	Ala	Lys	Ala	Glu	Gln	Gln	Ala	Gln	Glu	Asp	595	600	605	
Glu	Glu	Pro	Glu	Trp	Arg	Gly	Gly	Ala	Val	Gln	Pro	Pro	Arg	Asp	Gly	610	615	620	
Val	Leu	Glu	Pro	Lys	Ala	Ala	Lys	Glu	Gln	Pro	Lys	Ala	Gly	Lys	Glu				

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21

1-30. (canceled)

31. A method of inhibiting growth of a malignant cell comprising contacting the malignant cell with an agent effective to inhibit transport activity of RLIP76 in the malignant cell, wherein the malignant cell exhibits an increased expression level of RLIP76 protein relative to a non-malignant cell.

32. The method of claim **31**, wherein the agent is an antibody that binds specifically to the RLIP76 protein.

33. The method of claim **32**, wherein the antibody binds specifically to an epitope on the RLIP76 protein, wherein the epitope is expressed on the cell surface.

34. The method of claim **32**, wherein the antibody binds specifically to a region of RLIP76 consisting essentially of the amino acids of SEQ ID NO:3 or SEQ ID NO:4.

35. The method of claim **32**, wherein the antibody is in a composition comprising a pharmaceutically acceptable carrier.

36. The method of claim **35**, wherein the composition comprises a liposome.

37. The method of claim **31**, wherein the malignant cell is a melanoma cell, a small cell lung carcinoma cell, a non-small cell lung carcinoma cell, an ovarian cancer cell, a prostate cancer cell or an adenocarcinoma cell.

38. The method of claim **31**, wherein the malignant cell is a melanoma cell.

39. The method of claim **31**, wherein the malignant cell is in a subject.

40. The method of claim **31**, wherein the malignant cell is in a human subject.

41. The method of claim **31**, wherein the agent is a small molecule antagonist of the RLIP76 transport activity.

42. A method of inhibiting growth of a malignant cell, wherein the malignant cell exhibits an increased expression level of RLIP76 protein relative to a non-malignant cell, the method comprising contacting the cell with a composition that inhibits expression of RLIP76.

43. The method of claim **42**, wherein the composition comprises an anti-sense nucleic acid that is effective to inhibit expression of RLIP76 in the malignant cell.

44. The method of claim **43**, wherein the anti-sense nucleic acid is in a composition comprising a pharmaceutically acceptable carrier.

45. The method of claim **44**, wherein the composition comprises a liposome.

46. The method of claim **44**, wherein the composition comprises a proteoliposome comprising an RLIP76 protein.

47. The method of claim **42**, wherein the malignant cell is a melanoma cell, a small cell lung cancer cell, a non-small cell lung cancer cell, an ovarian cancer cell, a prostate cancer cell or an adenocarcinoma cell.

48. The method of claim **42**, wherein the malignant cell is a melanoma cell.

49. The method of claim **42**, wherein the cell is in a subject.

50. The method of claim **42**, wherein the cell is in a human subject.

51. The method of claim **42**, wherein the composition comprises a double stranded siRNA molecule that is effective to inhibit expression of RLIP76 in the malignant cell.

52. A method of inhibiting growth of a malignant cell in a subject, wherein the malignant cell exhibits an increased expression level of RLIP76 protein relative to a non-malignant cell, said method comprising:

administering to the subject a pharmaceutically acceptable composition comprising an effective amount of an anti-RLIP76 antibody to inhibit RLIP76 transport activity when bound to RLIP76 in a cell.

53. The method of claim **52**, wherein the antibody binds specifically to an epitope on the RLIP76 protein, wherein the epitope is expressed on the cell surface.

54. The method of claim **52**, wherein the antibody binds specifically to a region of RLIP76 consisting essentially of the amino acids of SEQ ID NO:3 or SEQ ID NO:4.

55. The method of claim **52**, wherein the pharmaceutically acceptable composition comprises a liposome.

56. The method of claim **52**, wherein the pharmaceutically acceptable composition comprises a proteoliposome comprising an RLIP76 protein.

57. The method of claim **52**, wherein the malignant cell is a melanoma cell, a small cell lung carcinoma cell, a non-small cell lung carcinoma cell, an ovarian cancer cell, a prostate cancer cell or an adenocarcinoma cell.

58. The method of claim **52**, wherein the malignant cell is a melanoma cell.

59. A method of inhibiting growth of a malignant cell in a subject comprising:

administering to the subject a pharmaceutically acceptable composition comprising an effective amount of an anti-sense nucleic acid that is effective to inhibit expression of RLIP76 in the malignant cell.

60. The method of claim **59**, wherein the anti-sense nucleic acid is complementary to an mRNA that encodes SEQ ID NO:22.

61. The method of claim **59**, wherein the pharmaceutically acceptable composition comprises a liposome.

62. The method of claim **59**, wherein the pharmaceutically acceptable composition comprises a proteoliposome comprising an RLIP76 protein.

63. The method of claim **59**, wherein the malignant cell is a melanoma cell, a small cell lung cancer cell, a non-small cell lung cancer cell, an ovarian cancer cell, a prostate cancer cell or an adenocarcinoma cell.

64. The method of claim **59**, wherein the malignant cell is a melanoma cell.

* * * * *