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(19) **United States**(12) **Patent Application Publication****Aswasthi et al.**(10) **Pub. No.: US 2017/0059556 A1**(43) **Pub. Date: Mar. 2, 2017**(54) **THERAPIES FOR CANCER USING RLIP76**(71) Applicant: **Board of Regents, The University of Texas System**, Austin, TX (US)(72) Inventors: **Sanjay Aswasthi**, Arlington, TX (US);
Sharad S. Singhal, Arlington, TX (US);
Sushma Yadav, Arlington, TX (US)(73) Assignee: **Board of Regents, The University of Texas System**, Austin, TX (US)(21) Appl. No.: **15/348,836**(22) Filed: **Nov. 10, 2016****Related U.S. Application Data**

(60) Division of application No. 14/622,738, filed on Feb. 13, 2015, which is a continuation of application No. 14/055,739, filed on Oct. 16, 2013, now abandoned, which is a 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.

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G01N 33/50 (2006.01)(52) **U.S. Cl.**
CPC **G01N 33/5011** (2013.01); **G01N 2333/705** (2013.01)(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.

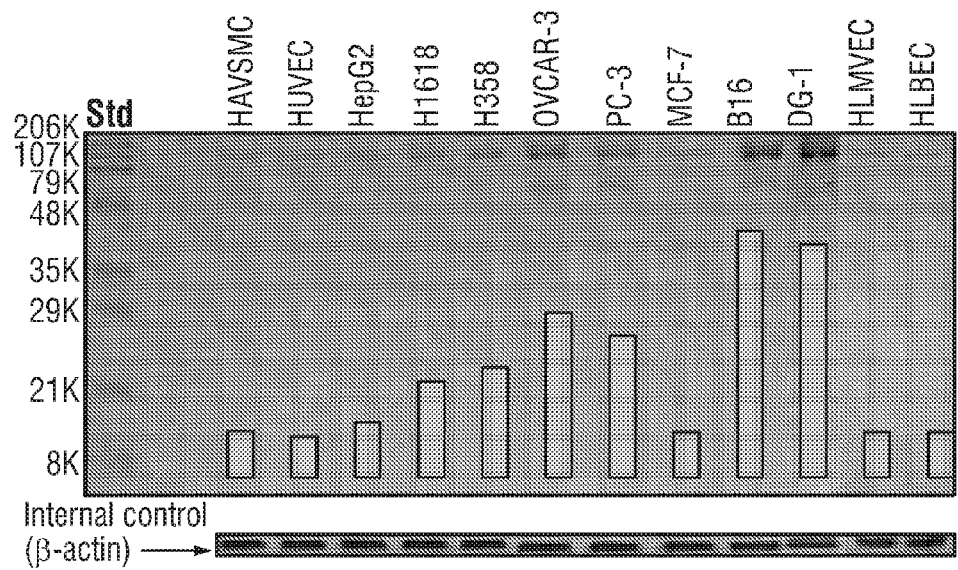


FIG. 1

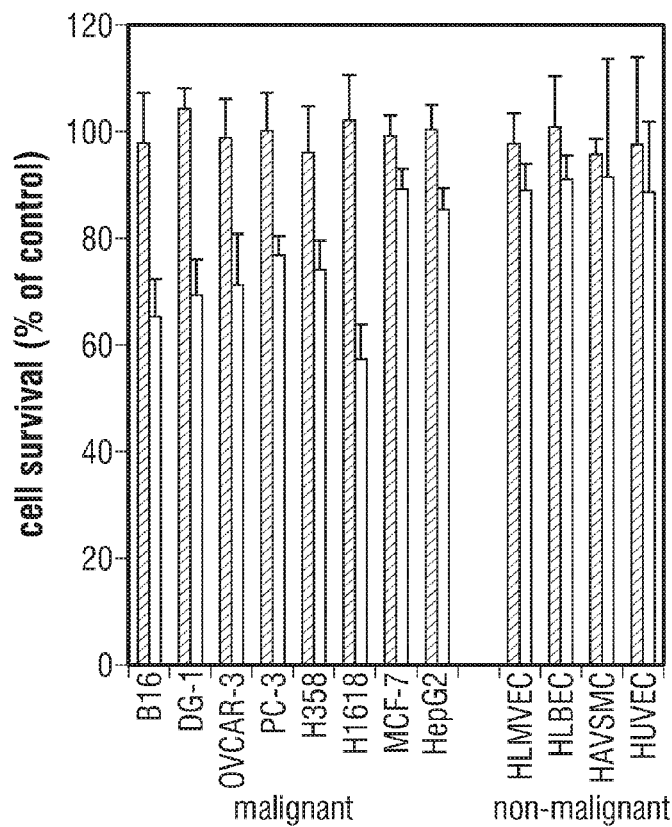
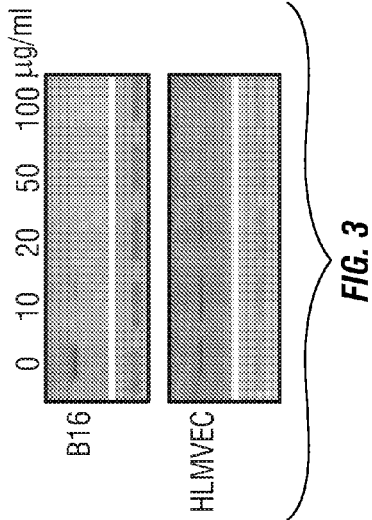
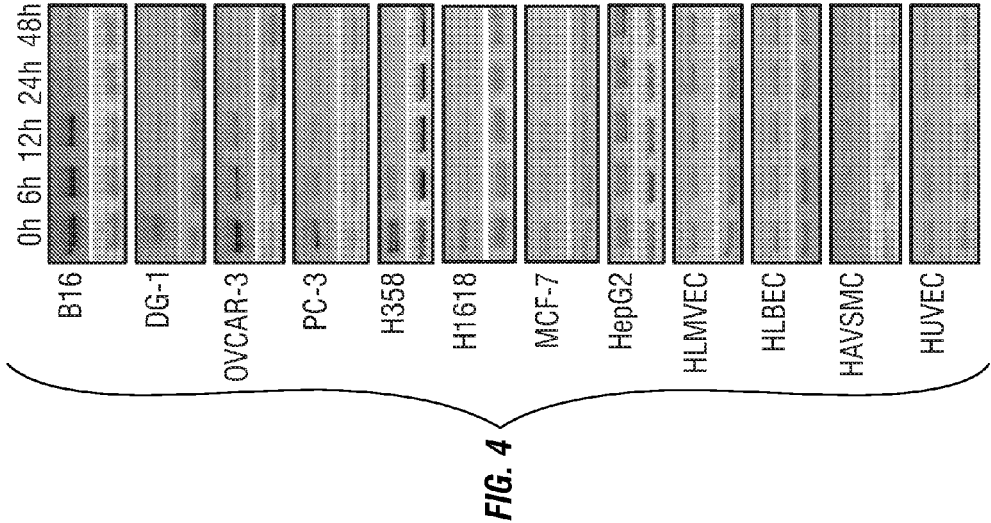
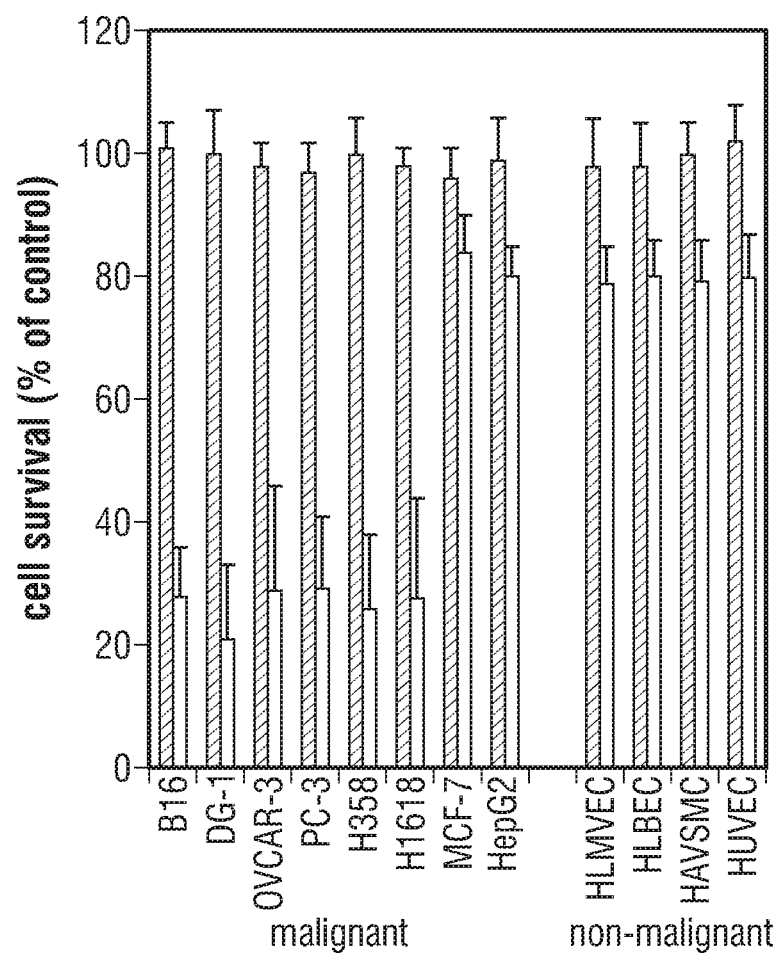
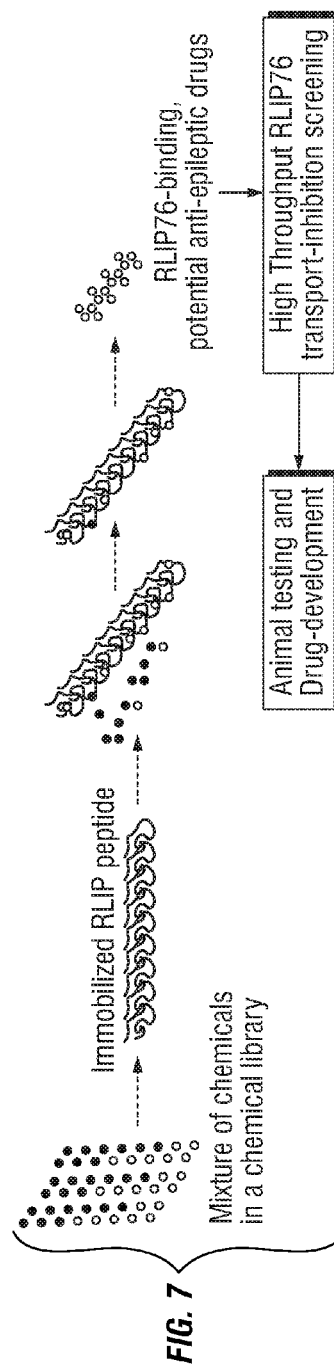
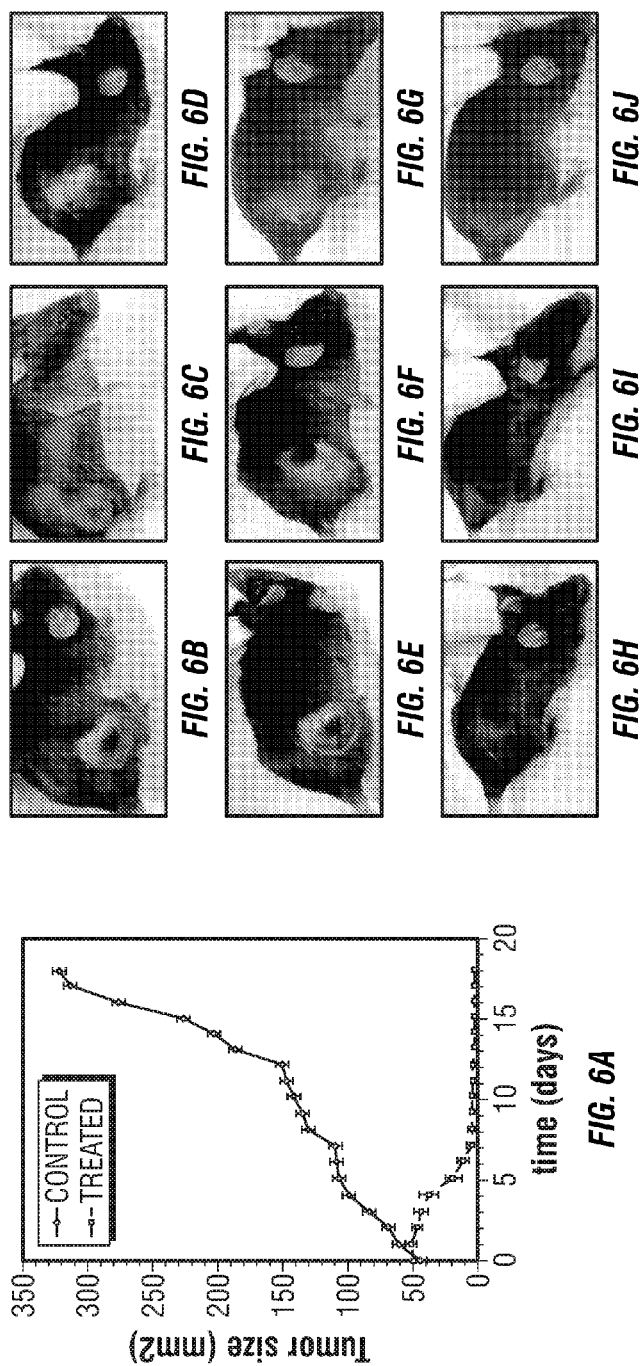


FIG. 2



**FIG. 5**



THERAPIES FOR CANCER USING RLIP76

CROSS-REFERENCES 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) 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. 6A 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] 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 for 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 RalBPL1. 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 (OVCAR-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 (HL-BEC), 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 μ g) 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 KH_2PO_4 , 0.3 mM Na_2HPO_4 , 4 mM NaHCO_3 , 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 PMSF, 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	
	$\mu\text{g}/10^8$	% of total	(pmol/min/mg)	
	cells	protein	DOX	DNP-SG
MALIGNANT CELLS				
B16 (mouse melanoma)	71 $\pm$ 6	0.8	443 $\pm$ 34	1796 $\pm$ 145
DG-1 (human melanoma)	63 $\pm$ 5	0.8	384 $\pm$ 28	1562 $\pm$ 112
OVCAR-3 (human ovary)	53 $\pm$ 3	0.7	298 $\pm$ 23	1194 $\pm$ 122
PC-3 (human prostate)	46 $\pm$ 3	0.6	211 $\pm$ 26	893 $\pm$ 66
H358 (human lung, NSCLC)	36 $\pm$ 3	0.6	180 $\pm$ 15	695 $\pm$ 56
H1618 (human lung, SCLC)	32 $\pm$ 3	0.5	96 $\pm$ 12	361 $\pm$ 41
MCF-7 (human breast)	15 $\pm$ 1	0.2	36 $\pm$ 3	105 $\pm$ 7
HepG2 (human liver)	17 $\pm$ 1	0.3	55 $\pm$ 5	167 $\pm$ 14
NON-MALIGNANT CELLS				
HLMVEC (human lung endothelium)	19 $\pm$ 2	0.3	40 $\pm$ 5	136 $\pm$ 14
HLBEC (human lung epithelium)	22 $\pm$ 2	0.4	46 $\pm$ 4	150 $\pm$ 20
HAVSM (human aorta smooth muscle)	15 $\pm$ 1	0.3	40 $\pm$ 6	125 $\pm$ 10
HUVEC (human umbilical endothelial)	14 $\pm$ 1	0.2	36 $\pm$ 4	113 $\pm$ 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 ^{14}C -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 μ g protein added to a 0.5 mL reaction mixture (50 mM Tris-HCl pH 7.4, 10 mM MgCl_2 , 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 - ^{32}P -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 $\mu\text{g}/\text{mL}$ 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 2×10^4 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 $\mu\text{g}/\text{mL}$ 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 IC_{50} . Measured absorbance values were directly linked with a spreadsheet for calculation of IC_{50} , 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 $\mu\text{g}/\text{mL}$), 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 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ RLIP76 si-RNA⁵⁰⁸⁻⁵²⁸ and performing Western-blot analyses after varying time of exposure. 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 $\mu\text{g/ml}$ final concentration) for about 24 hours. For floating cells, approximately 1×10^6 cells grown for 24 hours in 12-well plates then incubated with either pre-immune serum or anti-RLIP76 IgG (37 $\mu\text{g/ml}$ 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 mm^2 (day 11), animals were injected intraperitoneally with 100 μl diluent alone (phosphate buffered saline, PBS) or the same volume of diluent containing 200 μg 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 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 RLIP76⁵⁰⁸⁻⁵²⁸ phosphorothioate antisense oligonucleotide). Each of 21 animals was injected with 2×10^6 B16 mouse melanoma cell suspensions in 100 μl of PBS, subcutaneously. Animals were examined daily for signs of tumor growth. Treatment was administered when the tumor surface area exceeded 45 mm^2 (at day 11). Treatment consisted of 200 μg in 100 μl PBS of either anti-RLIP76 IgG, RLIP76⁵⁰⁸⁻⁵²⁸ si-RNA, or RLIP76⁵⁰⁸⁻⁵²⁸ phosphorothioate antisense oligonucleotide. Control groups were 200 $\mu\text{g}/100$ μl 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' GCGGATCCATGACTGAGTGCTTCCT (SEQ ID NO:5: BamHI restriction site is underlined) and down-

stream 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' GTAGAGAGGACCATGGTAGAGAAGTATGGC 3' (SEQ ID NO: 7) with its reverse complement); for del 154-171: 5' AAGAAGTCAAAAGACAAGCCAATTCAGGAG (SEQ ID NO: 8; with its reverse complement); for del 171-185: 5' GAAGAAAAAGAACTCAAACCCATTTTT 3' (SEQ ID NO: 9; with its reverse complement); for del 154-219: 5' GAAGAAGTCAAAAGACGTAGAGAAGTATGGC 3' (SEQ ID NO: 10; with its reverse complement); for del 415-448: 5' GAATTGTTTACATCGACAGGAGTGTGAAACC (SEQ ID NO: 11; with its reverse complement); and for del 65-80: 5' GTGTCTGATGATAGGACTGAAGGCTATG 3' (SEQ ID NO: 12 and its reverse complement).

[0046] 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 as 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 as 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 par-

tititioning 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 3.2×10^5 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 154-219, 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. Immuno-histochemistry 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 GGCUCUGAAUUGGCUUUUUCdTdT (SEQ ID NO: 15). The sequences of the scrambled si-RNA in the sense and antisense directions were GUAACUGCAACGAUUUCGAUGdTdT (SEQ ID NO: 16) and CAUCGAAUUCGUUGCAGUUACdTdT (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° 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., GGCTCCTGAATTGGCTTTTTC; 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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ccaggcattg	caattgacag	acatttgcct	ttcatagcgt	aaatgaggac	tcagcacagc	4243
caaccataat	cagcatgtct	gggatagact	ggtctagaat	aaaaatgaag	ttccatttgc	4303
tttgtttgct	ttaaaaattc	cacaattaaa	atatctgtca	ttgaaagctt	aaaaaaaaaa	4363
aaaaa						4368

<210> SEQ ID NO 2

<211> LENGTH: 655

<212> TYPE: PRT

<213> ORGANISM: homo sapiens

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<400> SEQUENCE: 2

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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
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 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

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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
Glu Asp Val Ser Lys Glu Glu Met Asn Glu Asn Glu Glu Val Ile Asn	465	470	475
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
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
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			

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<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
<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

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<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

<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:

-continued

<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
<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

caucgaauc 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

aagaaaagcc 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

-continued

<400> SEQUENCE: 20

```

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

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<210> SEQ ID NO 21

<211> LENGTH: 45

<212> TYPE: DNA

<213> ORGANISM: artificial sequence

<220> FEATURE:

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

<400> SEQUENCE: 21

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aagccaattc aggagccaga ggtgcctcag attgatgttc caaat                        45

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<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

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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 Gln Glu Asp 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

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Lys	Gln	Trp	Lys	Glu 165	Lys	Lys	Lys	Lys	Lys	Pro	Ile	Gln	Glu	Pro		
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	
					230					235					240	
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	tggt	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	tggt	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	
				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	gggt	ggg	ata	aag	gat	ttg	tct	aaa	gaa	gaa	aga	tta	tggt	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

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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	
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 tgc ccc agc agg gat agg aag gag acg tcc atc tga		1968
Pro Ala Lys Pro Ser Pro Ser Arg Asp Arg Lys Glu Thr Ser Ile		
	645 650 655	

<210> SEQ ID NO 23

<211> LENGTH: 655

<212> TYPE: PRT

<213> ORGANISM: homo sapiens

<400> SEQUENCE: 23

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	
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 115	Lys 130	Pro 115	Ser 115	Phe 115	Ser 120	Lys 120	Lys 120	Glu 120	Lys 125	Asp 125	Phe 125	Lys 130	Ile 135	Lys 140
Glu 145	Lys 145	His 145	Lys 150	Glu 150	Lys 150	Ser 155	Lys 160	Asp 165	Leu 170	Thr 175	Ala 180	Ala 185	Asp 190	Val 195
Lys 195	Gln 195	Trp 200	Lys 205	Glu 210	Lys 215	Lys 220	Lys 225	Lys 230	Lys 235	Lys 240	Pro 245	Ile 250	Gln 255	Glu 260
Glu 265	Val 270	Pro 275	Gln 280	Ile 285	Asp 290	Val 295	Pro 300	Asn 305	Leu 310	Lys 315	Pro 320	Ile 325	Phe 330	Gly 335
Pro 340	Leu 345	Ala 350	Asp 355	Ala 360	Val 365	Glu 370	Arg 375	Thr 380	Met 385	Met 390	Tyr 395	Asp 400	Gly 405	Ile 410
Leu 415	Pro 420	Ala 425	Val 430	Phe 435	Arg 440	Glu 445	Cys 450	Ile 455	Asp 460	Tyr 465	Val 470	Glu 475	Lys 480	Tyr 485
Met 490	Lys 495	Cys 500	Glu 505	Gly 510	Ile 515	Tyr 520	Arg 525	Val 530	Ser 535	Gly 540	Ile 545	Lys 550	Ser 555	Lys 560
Asp 565	Glu 570	Leu 575	Lys 580	Ala 585	Ala 590	Tyr 595	Asp 600	Arg 605	Glu 610	Glu 615	Ser 620	Thr 625	Asn 630	Leu 635
Asp 640	Tyr 645	Glu 650	Pro 655	Asn 660	Thr 665	Val 670	Ala 675	Ser 680	Leu 685	Leu 690	Lys 695	Gln 700	Tyr 705	Leu 710
Asp 715	Leu 720	Pro 725	Glu 730	Asn 735	Leu 740	Leu 745	Thr 750	Lys 755	Glu 760	Leu 765	Met 770	Pro 775	Arg 780	Phe 785
Glu 790	Ala 795	Cys 800	Gly 805	Arg 810	Thr 815	Thr 820	Glu 825	Thr 830	Glu 835	Lys 840	Val 845	Gln 850	Glu 855	Phe 860
Arg 865	Leu 870	Leu 875	Lys 880	Glu 885	Leu 890	Pro 895	Glu 900	Cys 905	Asn 910	Tyr 915	Leu 920	Leu 925	Ile 930	Ser 935
Leu 940	Ile 945	Val 950	His 955	Met 960	Asp 965	His 970	Val 975	Ile 980	Ala 985	Lys 990	Glu 995	Leu 1000	Glu 1005	Thr 1010
Met 1015	Asn 1020	Ile 1025	Gln 1030	Asn 1035	Ile 1040	Ser 1045	Ile 1050	Val 1055	Leu 1060	Ser 1065	Pro 1070	Thr 1075	Val 1080	Gln 1085
Ser 1090	Asn 1095	Arg 1100	Val 1105	Leu 1110	Tyr 1115	Val 1120	Phe 1125	Phe 1130	Thr 1135	His 1140	Val 1145	Gln 1150	Glu 1155	Leu 1160
Gly 1165	Asn 1170	Val 1175	Val 1180	Leu 1185	Lys 1190	Gln 1195	Val 1200	Met 1205	Lys 1210	Pro 1215	Leu 1220	Arg 1225	Trp 1230	Ser 1235
Met 1240	Ala 1245	Thr 1250	Met 1255	Pro 1260	Thr 1265	Leu 1270	Pro 1275	Glu 1280	Thr 1285	Gln 1290	Ala 1295	Gly 1300	Ile 1305	Lys 1310
Glu 1315	Ile 1320	Arg 1325	Arg 1330	Gln 1335	Glu 1340	Phe 1345	Leu 1350	Leu 1355	Asn 1360	Cys 1365	Leu 1370	His 1375	Arg 1380	Asp 1385
Gln 1390	Gly 1395	Gly 1400	Ile 1405	Lys 1410	Asp 1415	Leu 1420	Ser 1425	Lys 1430	Glu 1435	Glu 1440	Arg 1445	Leu 1450	Trp 1455	Glu 1460
Gln 1465	Arg 1470	Ile 1475	Leu 1480	Thr 1485	Ala 1490	Leu 1495	Lys 1500	Arg 1505	Lys 1510	Leu 1515	Arg 1520	Glu 1525	Ala 1530	Lys 1535
Gln 1540	Glu 1545	Cys 1550	Glu 1555	Thr 1560	Lys 1565	Ile 1570	Ala 1575	Gln 1580	Glu 1585	Ile 1590	Ala 1595	Ser 1600	Leu 1605	Ser 1610
Glu 1615	Asp 1620	Val 1625	Ser 1630	Lys 1635	Glu 1640	Glu 1645	Met 1650	Asn 1655	Glu 1660	Asn 1665	Glu 1670	Glu 1675	Val 1680	Ile 1685
Ile 1690	Leu 1695	Leu 1700	Ala 1705	Gln 1710	Glu 1715	Asn 1720	Glu 1725	Ile 1730	Leu 1735	Thr 1740	Glu 1745	Gln 1750	Glu 1755	Leu 1760
Leu 1765	Ala 1770	Met 1775	Glu 1780	Gln 1785	Phe 1790	Leu 1795	Arg 1800	Arg 1805	Gln 1810	Ile 1815	Ala 1820	Ser 1825	Glu 1830	Lys 1835
Glu 1840	Ile 1845	Glu 1850	Arg 1855	Leu 1860	Arg 1865	Ala 1870	Glu 1875	Ile 1880	Ala 1885	Glu 1890	Ile 1895	G		

-continued

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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		
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Val Leu Glu Pro Lys Ala Ala Lys Glu Gln Pro Lys Ala Gly Lys Glu		
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Pro Ala Lys Pro Ser Pro Ser Arg Asp Arg Lys Glu Thr Ser Ile		
645	650	655

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 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
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 <223> OTHER INFORMATION: scrambled sequence used with phosphorothioated DNA

<400> SEQUENCE: 24

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What is claimed is:

1. A method of screening for compounds that inhibit RLIP76 activity comprising contacting RLIP76 or a cell expressing RLIP76 with a compound to be tested and determining whether the compound inhibits the activity of RLIP76.

2. The method of claim 1, wherein contacting comprises contacting the compound to be tested with a sequence selected from the group consisting of SEQ ID NO:3-19 or SEQ ID NO:21.

3. The method of claim 1, wherein the RLIP76 comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:22 or SEQ ID NO:23.

4. The method of claim 1, wherein the determining comprises determining GSH conjugate activity.

5. The method of claim 1, wherein the determining comprises determining the transport activity of RLIP76.

6. The method of claim 2, wherein the transport of 4HNE is measured.

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