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Broad Distribution of the Multidrug Resistance-Related Vault Lung Resistance Protein in Normal Human Tissues and Tumors

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Multidrug resistance (MDR) to anticancer drugs is a major cause of treatment failure in cancer. The lung resistance protein LRP is a newly described protein related to MDR in several* in vitro *models. LRP has been shown to be a strong predictor of poor response to chemotherapy and prognosis in acute myeloid leukemia and in ovarian carcinoma patients. Recently, based on a 57% and 88% amino acid identity with major vault proteins from* Dictyostelium discoideum *and* Rattus norvegicus*, respectively, we identified LRP as the human major vault protein, the main component of highly conserved cellular organelles named vaults. We have studied the immunohistochemical expression of LRP in freshly frozen normal human tissues and 174 cancer specimens of 28 tumor types. LRP was broadly distributed in normal and malignant cells, but distinct patterns of expression were noticed. High LRP expression was seen in bronchus, digestive tract, renal proximal tubules, keratinocytes, macrophages, and adrenal cortex, whereas varying levels were observed in other organs. LRP was detected in all tumor types examined, but its frequency varied, fairly reflecting the chemosensitivity of different cancers. For example, low rates of LRP positivity were seen in testicular cancer, neuroblastoma, and acute myeloid leukemia; intermediate in ovarian cancer; and high in colon, renal, and pancreatic carcinomas. The wide occurrence of LRP in normal and transformed cells in humans, its similar distribution to that of vaults in other species, as well as the high level of conservation among eukaryotic cells of both the amino acid sequence of the major vault protein and the composition and structure of vaults, suggest that vault function is important to eukaryotic cells. (Am J Pathol 1996, 148:877–887)

Broad resistance to chemotherapeutic agents is a major cause of failure of cancer treatment. *In vitro* this phenomenon can occur as a result of exposure of cancer cells to a single cytotoxic drug and is called multidrug resistance (MDR).[1] MDR has been associated with the overexpression of *P*-glycoprotein (Pgp) or MDR-associated protein (MRP).[1–3] Pgp and MRP are ATP-binding-cassette transporters and function as efflux pumps decreasing intracellular drug accumulation, thereby conferring resistance to various natural product drugs.[1–3] However, the existence of MDR cell lines without Pgp or MRP expression indicates that other MDR mechanisms can be operative.[4,5]

We raised the monoclonal antibody (MAb) LRP-56 against the MDR lung cancer cell line SW-1573/2R120.[6] In SW-1573/2R120 cells LRP-56 specifically detected a ≈110 kd protein named lung resistance-related protein (LRP).[6] LRP was found to be overexpressed in several other MDR cell lines of different histogenetic origin,[6,7] some of them lacking Pgp or MRP expression.[5] Reversal of resistance in SW-1573/2R120 cells resulted in a parallel LRP decrease, further supporting the close association of LRP with drug resistance in these cells.[6]

Recently, we cloned the *LRP* gene.[8] Subsequent analysis of the deduced amino acid sequence indicated that the *LRP* gene product is a 100 kd protein showing 57% and 88% amino acid identity with the major vault protein α from the lower eukaryote *Dictyo-*

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stelium discoideum and with the major vault protein from the rat *Rattus norvegicus*, respectively.[8–10] Rat vaults are ribonucleoprotein particles described by Kedersha and Rome in 1986[11,12] that contain a major vault protein of 104 kd, which accounts for >70% of the particle, three minor proteins of 210, 192, and 54 kd, and a small RNA molecule.[11,12] These components are assembled in a complex barrel-shaped structure of approximately 57 times 32 nm with a nuclear mass of about 13 Mda, composing the largest ribonucleoprotein body reported to date: three times the size of a ribosome. Most vaults are present in the cytoplasm and a small fraction (~5%) at the nuclear pore complexes (NPCs).[12,13] NPCs mediate the bidirectional nucleocytoplasmic transport of a wide variety of molecules.[14] Structural similarities suggest that vaults form the central plug or transporter unit of NPCs, therefore implicating vaults in nucleocytoplasmic transport.[13] Cytoplasmic vaults were isolated associated with coated vesicles, but their exact structural and functional connection is unclear.[11,12] The fact that vaults from species as phylogenetically diverse as amoebas, amphibians, avians, and mammals are nearly identical in composition and structure suggests that their function is essential to eukaryotic cells.[12,15]

In addition to MDR-related proteins (Pgp, MRP, LRP), specific mechanisms of drug resistance have been documented *in vitro*. These include increased activity of the detoxification enzymes glutathione *S*-transferases (GST) and the DNA repair enzyme O^6-alkylguanine-DNA alkyltransferase, altered topoisomerase II activity, and overexpression of heavy metal-binding metallothioneins.[16,17] Resistance proteins are present in various normal tissues and their malignant counterparts. In normal cells, most of them may play a protective role against toxic compounds. Evidence suggests that the cells retain this function also in the malignant phenotype, and thus detoxify and eliminate anticancer drugs.[16]

In this study, we report the LRP distribution in normal human tissues and in a broad panel of tumors with dissimilar susceptibility to anticancer drugs. This information may be important to elucidate the significance of LRP and vaults in both normal and malignant phenotype, and its potential as predictor of response to chemotherapy.

Materials and Methods

Tissues

Normal human tissues obtained from adults and tumors were obtained from the Tissue Bank of the Pathology Laboratory, Free University Hospital, Amsterdam. Fresh tissues were snap-frozen in liquid nitrogen within 1 to 2 hours of resection and stored at −170°C until use. Several normal specimens from each organ site of different individuals (two to six samples) were used in the present study.

Monoclonal Antibody LRP-56

LRP-56 (immunoglobulin (Ig)G2b) was obtained after immunization of mice with SW-1573/2R120 cells, a Pgp-negative SW-1573 MDR subline.[6] By immunoprecipitation analysis using metabolically radiolabeled cells LRP-56 specifically detected a protein of ≈110 kd that was overexpressed in several MDR cell lines of different histogenetic origin.[6] LRP-56 also detected a ≈110 kd protein in MOP8 cells transfected with a full length cDNA of the *LRP* gene.[8]

Immunohistochemistry

Tissue sections were cut at 4 to 5 μm, thaw-mounted on poly-L-lysine-coated slides and dried overnight. For the study of tissues, fixation with acetone for 10 minutes at room temperature was used throughout. After fixation the slides were washed in phosphate-buffered saline (PBS) and preincubated with normal rabbit serum (1:50 in PBS plus 1% bovine serum albumin (Sigma Chemical Co., St. Louis, MO) for 10 minutes. Blocking serum was drained off and LRP-56 (1:500) was incubated for 1 hour. Sections were washed three times for 10 minutes with PBS and incubated for 30 minutes with affinity-purified rabbit anti-mouse IgG conjugated to horseradish peroxidase (1:25; DAKO Corp., Glostrup, Denmark). All incubations were performed at room temperature in humid chambers. Diaminobenzidine (DAB) (Sigma Chemical Co.) was used as chromogen. Slides were counterstained with hematoxylin. Cytospin preparations of tumor cell lines (non-small cell lung cancer SW-1573 and small cell lung cancer GLC4) and their corresponding MDR sublines overexpressing LRP (SW-1573/2R120 and GLC4/ADR) served as controls.[6] Negative control slides were treated as above, substituting LRP-56 with an irrelevant mouse MAb. In addition, some formalin and Sensofix-fixed (Sensomed, Beuningen, The Netherlands), paraffin-embedded normal tissues and tumors were tested. Deparaffinized sections were pretreated with 0.1% pepsin (Sigma Chemical Co.), 0.1% trypsin (Flow Laboratories), and tissue unmasking fluid (Sambio, Uden, The Netherlands), respectively. Then, an avidin-biotin peroxidase complex method was employed using the ABC Elite kit (Vector, Burlingame, CA). DAB was used as a chromogen.

Table 1. *LRP Expression in Acetone-fixed, Frozen Normal Human Tissues*

Tissue	LRP*	Tissue	LRP*
Skin		Female reproductive system,	
Keratinocytes	●●	Ovary *(continued)*	
Sweat glands	◒	Germinal cells	◒
Melanocytes	●	Rest (no vessels)	○
Lung		Endometrium	◒
Bronchial cells	●●	Placenta (trophoblast)	◒
Pneumocytes	◒	Nervous system	
Alveolar macrophages	●●	Neurons	○
Digestive tract epithelia		Purkinje cells	◒
Esophagus	●●	Glial cells	○
Stomach	●●	Peripheral nerves	○
Small intestine	●●	Hematopoietic/immune system	
Large intestine	●●	Thymus	
Liver		Epithelial cells	◒
Hepatocytes	◒	Lymphoid cells	○
Biliary ducts	◒	Spleen	
Bile canaliculi	○	White pulp	○
Pancreas		Red pulp	○
Ductal cells	●	Lymph nodes	
Acinar cells	◒	Lymphocytes	○
Urinary system		Macrophages	●●
Kidney		Peripheral blood	+†
Glomeruli	◒	Bone marrow	+†
Proximal tubules	●	Connective tissue	
Distal tubules	○	Smooth muscle	○
Urothelium		Skeletal muscle	○
Ureter	●	Fibroblasts	◒
Bladder	●	Chondrocytes	○
Endocrine system		Adypocites	○
Adrenal gland		Interstitial matrix	◒
Cortex	●●	Endothelial cells	
Medulla	○	Skin	◒
Thyroid	○	Lung	◒
Parathyroid	○	Esophagus	◒
Hypophysis	○	Stomach	◒
Male reproductive system		Large intestine	◒
Testes, germinal cells	●	Kidney glomeruli	◒
Prostate	◒	Prostate	◒
Female reproductive system		Testes	◒
Breast	◒	Uterus	◒
Ovary		Brain	◒
Epithelial cells	◒	Others (see text)	◒

*●●: Homogeneous staining with 90–100% of cells showing strong positivity; ●: Homogeneous staining with 90–100% of cells showing positivity; ◒: Heterogeneous staining with 10–90% of cells showing positivity; ○: negative.
†LRP expression detected by immunoprecipitation.

Immunoprecipitation

Fresh normal tissues obtained from adults and tumors were collected within 1 to 2 hours from surgery, minced, and immediately frozen. The cells were then mechanically disrupted in a Mikro-Dismembrator II (Salm & Kipp, Breukelen, The Netherlands) and homogenized in lysis buffer as described.[6] Nuclei and unsolubilized cell membranes were removed by centrifugation at 14,000 rpm for 10 minutes. Supernatant aliquots were diluted 1:1 to a total volume of 1 ml in lysis buffer supplemented with 1% nonfat milk powder and precleared with 100 μl protein A-Sepharose (CL-4B diluted 1:1 in PBS; Pharmacia, Uppsala, Sweden) to reduce nonspecific binding. Incubation with 15 μl of undiluted LRP-56 (1 mg/ml) or an equivalent amount of an irrelevant mouse MAb for 2 hours at 4°C was then performed. Immune complexes were precipitated by further incubation for 1 hour at 4°C with 100 μl protein A-Sepharose. Precipitates were washed twice with lysis buffer supplemented with 0.1% sodium dodecyl sulphate and four times with PBS, and resuspended in 50 μl sample buffer as described.[6] After centrifugation, supernatants were loaded for overnight polyacrylamide (4 to 12%) gel electrophoresis (8 mA). Gels were stained with Coomassie Brilliant Blue (Pharmacia). Molecular weight standards were from Bio-Rad Laboratories (Richmond, CA).

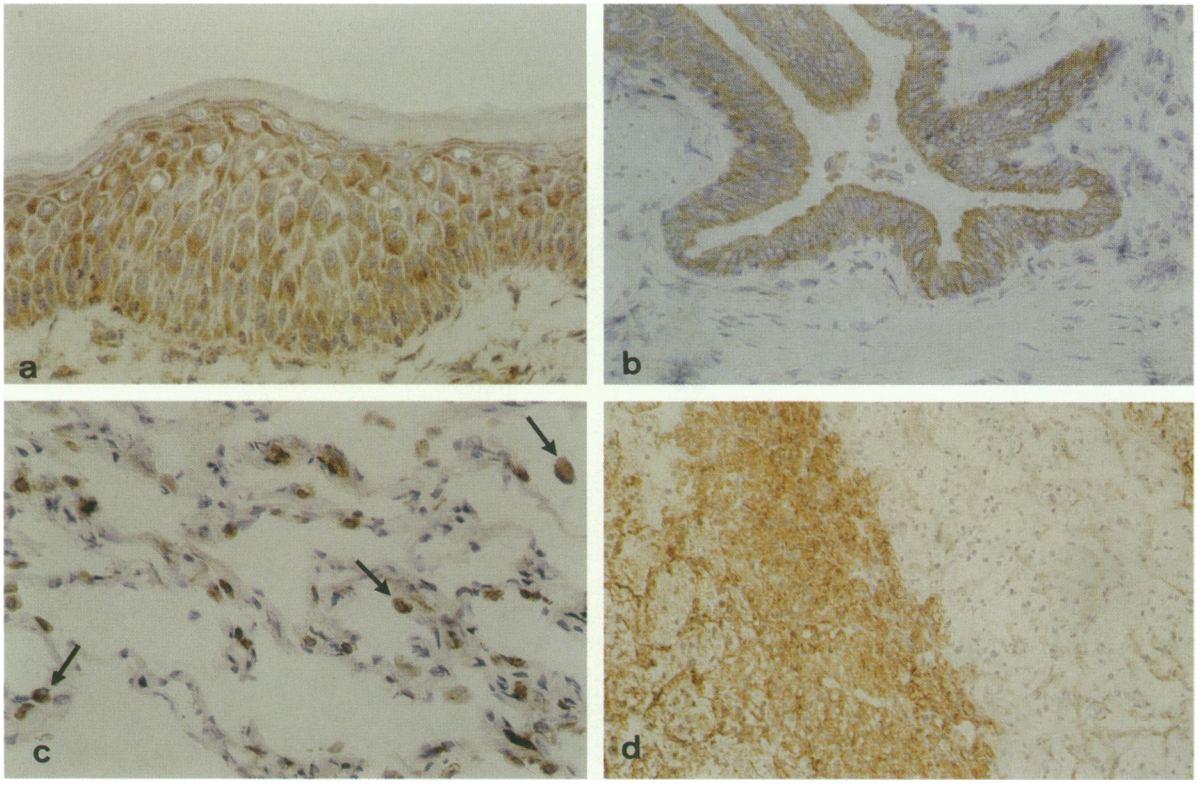

Figure 1. *LRP expression in normal human tissues as detected by staining of frozen tissue sections with LRP-56 (immunoperoxidase). Strong immunoreactivity was observed in the majority of* (a) *keratinocytes* (×63), (b) *bronchial epithelia* (×40), (c) *alveolar macrophages* (small arrows) (×40), (d) *adrenal cortex (left half)* (×20).

Results

LRP was widely distributed in both normal human tissues and tumors. In all the instances of LRP positivity reported in this study, the negative control slides incubated with an irrelevant mouse IgG were negative. LRP-56 showed a characteristic cytoplasmic granular immunoreactivity. Optimum staining results were obtained when LRP-56 was applied to cryostat sections fixed with acetone for 10 minutes. No immunoreactivity was seen when ethanol or methanol was used as a fixative. Immunoreactivity was low in formalin-fixed, paraffin-embedded tissues, although the staining intensity could be enhanced by pretreatment with tissue unmasking fluid.

LRP Expression in Normal Human Tissues

Table 1 summarizes the detailed distribution of LRP in frozen, acetone-fixed, normal human tissues. LRP-56 staining was evaluated in a semiquantitative fashion as indicated in the footnote to Table 1.

Skin

LRP-56 showed strong immunoreactivity with epidermal keratinocytes and melanocytes (Figure 1a). Excretory ducts of sweat glands were also positive, although the staining was heterogeneous and not as intense as in keratinocytes. The secretory portions of sweat glands were unreactive. Some mesenchymal dermal cells, likely fibroblasts and macrophages, showed LRP immunoreactivity (Figure 1a).

Lung

Epithelial cells lining the bronchioles stained strongly (Figure 1b). Type I and II pneumocytes showed patchy immunoreactivity, whereas alveolar macrophages were strongly positive (Figure 1c).

Digestive Tract

Stratified squamous epithelium of the esophagus was strongly positive, and positive reactivity of some mucous glands was also noted. Surface epithelial cells lining the lumen of the stomach, small intestine, and colon showed intense and diffuse staining; frequently the staining was stronger on the luminal portion of these cells. In the liver, hepatocytes were negative or faintly positive, bile canaliculi failed to stain, but the epithelium lining biliary ducts was positive. In the pancreas, the epithelial cells lining the

ducts were positive. Heterogeneous (in some specimens limited to a few glands) and weak immunoreactivity was observed in the pancreatic acini.

Urinary System

Strong staining was seen in the proximal tubules of the kidney. Distal tubules and collecting ducts were negative. Glomeruli showed LRP-56 immunoreactivity at capillary endothelial cells, but positivity of other glomerular cells could not be excluded. Transitional epithelial cells of the ureter and urinary bladder stained positively.

Endocrine Glands

In the adrenal, intense LRP-56 staining was detected in the cortex, the strongest reaction being observed in the zona reticularis, whereas cromaffin cells in the medulla were mostly unreactive (Figure 1d). Scattered LRP-56 positivity, compatible with staining of the vascular supporting stroma, was observed in the medulla, although positivity of occasional cromaffin cells cannot be excluded (Figure 1d). Epithelial cells lining thyroid follicles, as well as cells of the anterior pituitary and parathyroid had no detectable LRP-56 immunoreactivity. The islets of Langerhans were unreactive or showed faint positivity.

Male Reproductive System

Germ cells of the seminiferous tubules and Leydig cells showed positive staining. The prostatic glandular epithelium had a patchy immunoreactivity.

Breast and Female Reproductive System

Heterogeneous LRP-56 staining was detected in duct-lining cells of the breast, placental trophoblast, epithelial ovarian cells, and germ cells of some ovarian follicles.

Nervous Tissues

No detectable staining of neurons was observed, except for the Purkinje cells of the cerebellum. Glial cells of the brain and peripheral nerves were unreactive.

Hematopoietic/Immune System

Epithelial cells of the thymus were positive for LRP-56, but lymphocytes in the cortex and medulla were negative. White pulp cells of the spleen, and cortical and medullar lymphocytes of lymph nodes failed to stain. In the red pulp of the spleen and the follicular and paracortical areas of lymph nodes, some macrophages and dendritic cells were immunoreactive. Macrophages showed strong LRP immunoreactivity in different normal and malignant tissues. High expression of LRP in macrophages was confirmed using an alkaline phosphatase method.

Connective Tissue

Fibroblasts were occasionally positive, with variable staining intensity among different tissues and among different specimens within a particular tissue type. The extracellular matrix was mainly negative, but in some organs, like the lymph nodes, a matrix staining was detectable. Systematically, the staining of connective tissue elements persisted with further dilution of LRP-56 and was absent in the negative control slides incubated with an irrelevant mouse MAb. Smooth and striated muscle, chondrocytes, and adipocytes failed to stain.

Endothelial Cells

LRP-56 staining has been noted in endothelial cells in several organs, including testes, brain, lung, colon, liver, kidney glomeruli, thyroid, breast, ovary, myometrium, and lymph nodes.

LRP Expression in Human Cancers

Table 2 summarizes LRP expression in 174 tumor samples of 28 types of cancer, almost all of them being primary untreated tumors. Representative examples of staining are shown in Figure 2. In general, tumors were either completely LRP-negative (Figure 2a) or showed LRP immunoreactivity in the majority of cancer cells (Figure 2, b–d). However, in few instances predominantly negative tumors showed scattered LRP-positive malignant cells (<10%). In LRP-positive tumors, both homogeneous and heterogenous staining intensity could be observed in different specimens. Usually, LRP expression was higher in well differentiated tumoral areas. As in normal tissues, mesenchymal cells within the tumor specimen, most likely fibroblast and macrophages, frequently showed moderate to strong LRP staining. This feature was observed in both LRP-positive and LRP-negative tumors (Figure 2a).

Low frequency of LRP expression was seen in testicular cancer (germ cell tumors), Wilms' tumor,

Table 2. *LRP Expression in Human Cancers*

Cancer type	Number of samples	LRP-positive (≥10% cells)	LRP-positive (<10% cells)	LRP-negative
Testicular cancer	12	4	2*	6
Wilms' tumor†	7	1	2	4
Rhabdomyosarcoma	8	3*	1*	4
Neuroblastoma	6	0	2*	4‡
Ewing's sarcoma	6	1*	1	4
Acute myeloid leukemia	20	3	2	15
Small cell lung cancer	4	1	1	2
Ovarian carcinoma	20	15	4	1
Breast carcinoma	6	5	0	1
Bladder carcinoma	5	4	0	1
Head and neck carcinoma	7	6	0	1
Soft tissue sarcoma	7	5	2*	0
Osteosarcoma	3	2	0	1
Endometrial carcinoma	5	5	0	0
Gastric carcinoma	4	4	0	0
Esophageal carcinoma				
Squamous cell	3	3	0	0
Adenocarcinoma	1	1	0	0
Colo-rectal carcinoma	10	10	0	0
Pancreatic carcinoma	5	5	0	0
Renal carcinoma	7	7	0	0
Melanoma	3	3	0	0
Non-small cell lung cancer				
Squamous cell carcinoma	5	1	0	4
Adenocarcinoma	4	4	0	0
Mesothelioma	2	2	0	0
Pheochromocytoma	4	4	0	0
Thyroid carcinoma	5	4	0	1
Hepatocellular carcinoma	1	1	0	0
Adrenal carcinoma	4	4	0	0

*One sample studied in each case was refractory primary or metastastic tumor after chemotherapy (CT).
†Chemotherapy status unknown.
‡Two of the samples studied were primary tumor after preoperative CT (patients received CT as initial treatment after clinical diagnosis).

rhabdomyosarcoma, neuroblastoma, Ewing's sarcoma, and acute myeloid leukemias. In neuroblastomas, scattered mature ganglion cells showed strong LRP immunoreactivity. Of interest, a number of these tumors bearing LRP-positive cells were refractory tumors after chemotherapy treatment (Table 2, footnote *). A significant proportion of small cell lung cancers and, to a lesser extent, of ovarian carcinomas were LRP-negative or showed LRP immunoreactivity in few cancer cells (<10%). In contrast, the vast majority of other solid tumors were predominantly LRP-positive. For example, colo-rectal, renal, pancreatic, and endometrial carcinomas, among others, expressed LRP in virtually all the cases. Only a minority of specimens from cancers of the breast, bladder, head and neck, and thyroid, as well as sarcomas, showed none or low (<10% of tumor cells) LRP immunoreactivity. Adenocarcinomas of the lung were also mostly LRP-positive, whereas a high proportion of squamous cell carcinomas were LRP-negative. This is in contrast with esophageal and head and neck squamous cell cancers, which were mostly LRP-positive.

Immunoprecipitation

To further demonstrate the specificity of LRP-56 in normal human tissues and tumors, immunoprecipitation was performed (Figure 3). Western blotting could not be used, because LRP-56 does not detect denaturated protein.[6] LRP-56 reacted with a protein of ≈110 kd, not only in the 2R120 control cells, but also in several specimens analyzed including colon (n = 2), adrenal (n = 2), colon carcinoma (n = 2), ovarian carcinoma (n = 1), liver carcinoma (n = 1), and laryngeal carcinoma (n = 1). In addition, a specific ≈110 kd band was also detected in peripheral blood (n = 4) and bone marrow (n = 2) of normal donors (Table 1). In some samples, as in normal colon (Fig. 3), two close bands at ≈110 kd and ≈105 kd could be detected. An additional band in lanes denoted "b" and two bands in lanes denoted "a" are also present when only LRP-56 (lane 1) or an irrelevant mouse antibody (data not shown), respectively,

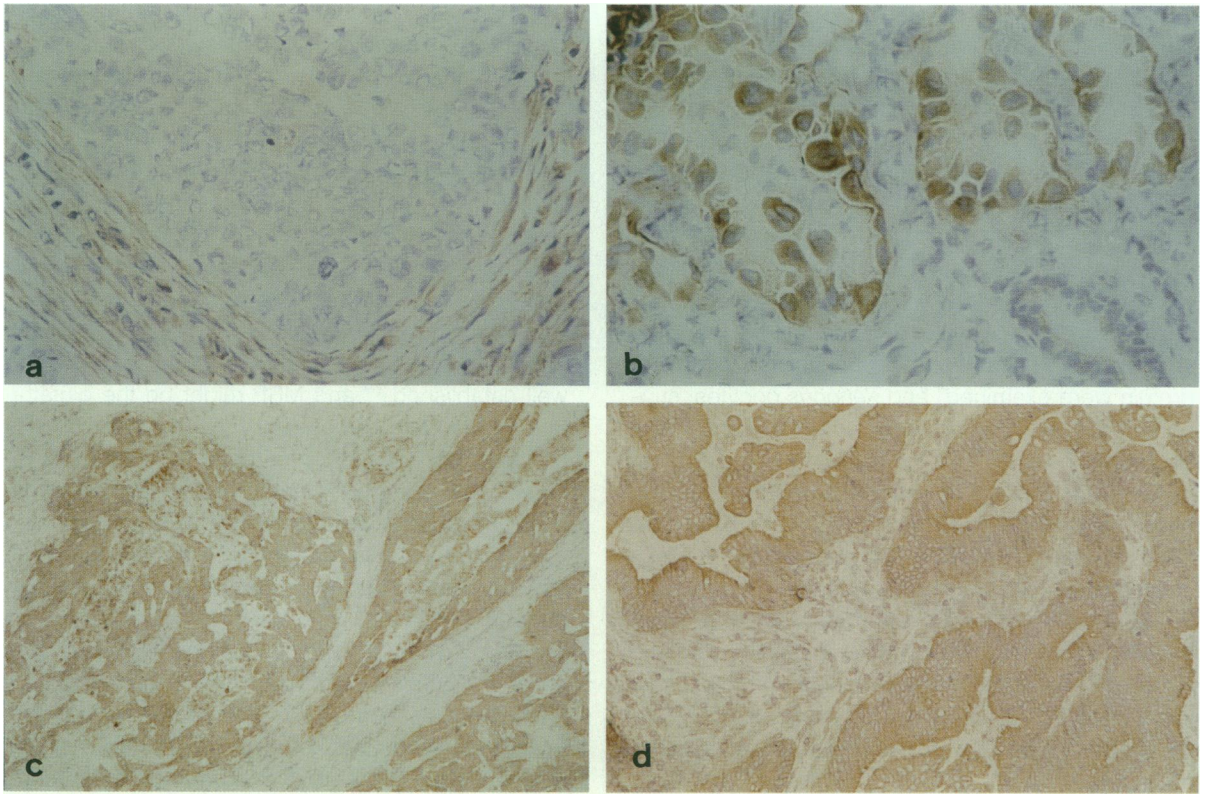

Figure 2. *LRP expression in human tumors as detected by staining of frozen tissue sections with LRP-56 (immunoperoxidase).* **(a)** *LRP-negative ovarian carcinoma. The mesenchymal cells surrounding the tumor show strong LRP immunoreactivity (×40).* **(b)** *Heterogeneous LRP expression in an ovarian carcinoma (×40).* **(c)** *Strong LRP-56 immunoreactivity in most of the tumor cells from an esophageal adenocarcinoma (×10).* **(d)** *Strong LRP-56 immunoreactivity in most of the tumor cells from a colon carcinoma (×20).*

were loaded in the absence of cell lysates, and are due to precipitation of the immunoglobulins themselves.

Discussion

In this study we found that the MDR-associated protein LRP, recently identified as the human major vault protein,[8] is widely distributed in normal human tissues and tumors, suggesting an ubiquitous role for vaults in normal and malignant cells.

LRP-56 specificity for the major vault protein was confirmed by the immunoprecipitation of a ≈110 kd band, which was also recognized by LRP-56 in SW-1573/2R120 control cells and in metabolically radio-

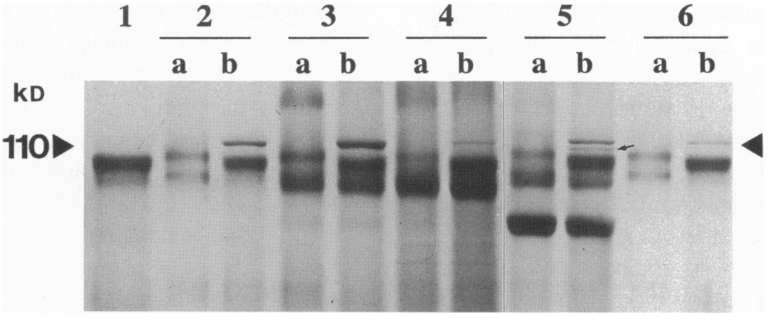

Figure 3. *Immunoprecipitation analysis with LRP-56 in normal tissues and tumors. Lysates were immunoprecipitated as described in Materials and Methods with LRP-56 (lanes denoted b) or with an irrelevant mouse antibody (lanes denoted a). LRP-56 recognizes a ≈110 kd protein (LRP,* **large arrows** *) in the non-Pgp MDR cell line SW-1573/2R120 used as a positive control (lane 2b), in normal adrenal (lane 3b), liver carcinoma (lane 4b), normal colon (lane 5b), peripheral blood mononuclear cells (lane 6b), and in other normal tissues and tumors tested as well (see text). Small arrow shows an additional band at ≈105 kd in normal colon (lane 5b). No 110 kd protein was detected by the negative control in the SW-1573/2R120 cell line and in the same tissues (lanes 2a to 6a, respectively). An additional band in lanes denoted b and two bands in lanes denoted a are also present when only LRP-56 (lane 1) or an irrelevant mouse antibody (data not shown), respectively, were loaded in the absence of cell lysates, and are due to precipitation of the immunoglobulins themselves.*

labeled MOP8 cells transfected with the LRP gene.[6,8] Antibodies raised against entire rat vaults were reported to be specific for the major vault protein, the immunodominant component of vaults, and their immunoreactivity related to the total amount of vaults.[11,15] Thus, LRP-56 immunoreactivity most likely represents vault distribution, as well. In some tissues (eg, normal colon) LRP was resolved as a doublet, although the smaller species was weakly recognized by LRP-56. A similar doublet has been reported in *Dictyostelium discoideum* (the smallest species was only detectable when large amounts of protein were examined) and *Xenopus* oocytes, supporting the view that different isoforms of the major vault protein exist.[15] LRP expression was cytoplasmic in all cell types examined, in agreement with the predominant location of vaults.[11,12] Lack of an evident nuclear rim staining, corresponding to NPC-associated vaults,[13] may be due to their relatively low abundance at the nuclear membrane and signal masking by cytoplasmic vaults. Based on striking structural similarities and their location at the NPCs, it has been strongly suggested that vaults constitute the plugs of the NPCs, thereby implicating vaults in nucleocytoplasmic transport processes.[13] Whether cytoplasmic vaults may also play a role in transport is unknown. Although no functional conclusions can be drawn from the present descriptive study, these data should provide clues to aid the further investigation of the biological role of vaults.

In normal tissues, high LRP expression was detected in epithelial cells from the bronchus and digestive tract, as well as in keratinocytes, macrophages and adrenal cortex. Relatively high expression was also found in proximal tubules of the kidney, transitional urothelium, ductal cells in the pancreas, and germ cells. Variable LRP reactivity was found in other organs as well as in fibroblasts. Importantly, also in other species vaults are abundantly expressed in epithelial cells and macrophages, eg, rat intestine and rabbit alveolar macrophages, and present in fibroblasts.[12,15] Thus, besides the high degree of structural and morphological conservation of vaults, their similar cellular distribution across evolutionary diverse species further supports the view that vaults are important to normal cellular function. In addition, we found LRP expression in cells in which the existence of vaults had not been studied before, including adrenal cortex, germ cells, trophoblast, melanocytes, Purkinje cells, and endothelial cells at different locations such as brain and testes blood barriers. The ubiquitous presence of LRP in such a broad panel of highly specialized cell types suggests that vaults fulfill a common basic function(s) in all cells, although cell type-specific roles cannot be excluded.

The distribution of LRP in normal tissues is remarkably similar to that of other drug-resistance related proteins. Moderate to high levels of Pgp, GST, and MRP are also present in epithelia lining the digestive and respiratory tracts, transitional epithelia (except Pgp), and macrophages.[18–21] MRP and GST are also highly expressed in keratinocytes. Based on these localizations, a defensive role against exogenous toxic compounds has been proposed for Pgp. The role of GST in detoxification processes has been well established,[20] and MRP might be the glutathione *S*-conjugate carrier described in bronchial cells and macrophages.[21] Since these cells share exposure to an enormous variety of toxic compounds, other molecules or cellular organelles might also contribute to cellular protection. In particular, vaults may contribute to defense against nuclear toxins. In support of this view, the LRP-overexpressing MDR cell line SW-1573/2R120 and other MDR cell lines show a decrease in the nuclear accumulation of fluorescent daunorubicin.[22] The molecular basis of this phenomenon has not been yet identified.

Like Pgp, LRP is highly expressed in adrenal, where there is also high MRP expression, and in endothelial cells at brain and testes blood barriers.[18–19] *In vitro* and *in vivo* models suggest that Pgp at the blood-brain barrier excludes xenobiotics, including natural product anticancer drugs, from central nervous system (CNS) tissues. The lack of CNS penetration of xenobiotics and drugs that do not interact with Pgp indicates that other molecules are functional components of these blood barriers. Significant differences between LRP and Pgp distribution include lack of LRP expression in bile canaliculi.[18,19]

Further parallelism between LRP and MRP tissue distribution was observed in many other types of cells, including epithelial cells of sweat glands, breast, prostate, endometrium, and hepatocytes and placental throphoblast.[21] However, LRP was not detected in muscle tissues, which contain significant levels of MRP. Similarly, additional analogy between LRP and GST expression in human tissues is also considerable, including moderate to high expression of both proteins in placenta trophoblast, liver biliary ducts, transitional epithelium, and pancreas.[20] This extensive overlapping of different drug-resistance-related proteins in normal human tissues suggests that they may fulfill complementary functions.

High vault immunoreactivity has been reported in rat glial cells during the late embryonic and early postnatal period. The expression of vaults progres-

sively diminished afterward, and only very weakly stained glial cells were observed in the adult rat.[23] Similarly, lack of LRP staining of brain glial cells in human adults indicates absence or very reduced amount of vaults. The possibility that the expression of vaults in brain glial cells or in other cell types might be differentially regulated during development deserved further investigation.

Data on the presence of vaults or the expression of LRP in cancer cells is limited.[6,12,15] Besides the overexpression of LRP in most Pgp-negative MDR cancer cell lines, we have recently reported that 78% of 59 drug-unselected cancer cell lines (including melanoma, leukemia, and colon, renal, small cell, and non-small cell lung, breast, ovarian, and brain cancers) used at the National Cancer Institute (NCI) for anticancer drug screening expressed LRP at varying levels.[24] Of interest, LRP expression was lower in leukemias and small cell lung cancer, whereas >90% of colon and renal cancer lines were LRP-positive. The data presented here confirm a similar widespread and differential LRP distribution in human cancers *in vivo*. In general, cells seem to preserve their LRP phenotype during transformation, a feature shared with other resistance-associated proteins such as Pgp, MRP, and GST. However, some tumor types expressed significantly higher levels of LRP than their normal counterparts (eg, thyroid carcinoma), whereas in others LRP expression seems to be downregulated during transformation (eg, germ cell tumors).

The actual contribution of vaults to the transformed phenotype is unknown. However, all presently available evidence indicates a close association between LRP expression in tumors and reduced susceptibility to anticancer agents. Drug-selected MDR sublines derived from both LRP-positive and -negative tumor cell lines showed LRP overexpression, and reversal of resistance led to a parallel LRP decrease.[6] In the NCI panel of 61 cancer cell lines, the expression of LRP significantly correlated with *in vitro* resistance to various structurally and functionally unrelated drugs, including doxorubicin, etoposide, cisplatin, carboplatin, and melphalan. Remarkably, LRP was a marker of drug resistance superior to Pgp or MRP in this model.[24]

Our present results show that the frequency of LRP expression was low in testicular germ cell tumors, Wilms' tumors, rhabdomyosarcoma, neuroblastoma, Ewing's sarcoma, acute myeloid leukemia, and small cell lung cancer. These tumors of dissimilar histogenetic origin are remarkably sensitive to chemotherapy with a high rate of complete responses (and in some cases of cures) in metastatic disease.[25] In contrast, most primary solid tumors showed LRP expression in the majority of specimens studied. For example, in cancers of the colon, pancreas, adrenal gland, and kidney, LRP was expressed in virtually all the specimens studied. The results of the treatment of these tumors with chemotherapy are poor in terms of complete responses and long term survival.[25] A number of ovarian carcinoma specimens were negative or showed LRP immunoreactivity in few occasional cells. Clinically, this cancer is relatively sensitive to chemotherapy with a high rate of responses, but only few patients with advanced disease show a long progression-free interval.[24]

Although the expression of LRP in a large panel of human tumors fairly reflects the responsiveness to chemotherapy of different tumor types, it may be argued that histogenetically dissimilar malignancies could show differential LRP expression for reasons unrelated to chemosensitivity. Therefore, the establishment of LRP as a marker of clinical drug resistance will require clinico-pathological studies in specific tumor types. To date, this sort of study has been performed in adult acute myeloid leukemia (AML) and in ovarian carcinoma. List et al[26] reported the expression of Pgp and LRP in 33 of 77 (43%) AML specimens, including de novo, secondary, and relapsed AML. Both Pgp and LRP expression were significantly associated with poor response to chemotherapy, but only LRP retained prognostic value in multivariate analysis.[26] We have studied Pgp, MRP, and LRP expression in 57 advanced ovarian carcinomas treated with cisplatin-based chemotherapy.[27] Of the tumors, 16 and 18% were Pgp- and MRP-positive, respectively. Pgp and MRP showed no prognostic value in this group of patients. Forty-four tumors (77%) expressed LRP, and this expression was significatively associated with poor response to chemotherapy, and shorter progression-free and overall survival.[27]

A number of MDR cancer cells have been shown to distribute anticancer drugs into the perinuclear region and, subsequently, redistribute the drug away from the nucleus into a punctate cytoplasmic pattern.[22,28] It is tempting to speculate that vaults may play a role in drug resistance by regulating both the nucleocytoplasmic transport and the cytoplasmic redistribution of drugs.

In conclusion, we described the broad distribution in normal and malignant tissues of the drug resistance-related protein LRP, the major component of human vaults. The high degree of conservation of vaults among phylogenetically very distant eukaryotic cells suggests an important role for vaults in cell

biology. The *in vitro* and clinical data accumulated so far point to LRP as a marker of resistance to anticancer drugs, although a functional role for vaults in such a process remains to be demonstrated. The ongoing functional characterization of vaults will clarify its role in normal and transformed cells. The results presented here may contribute to assist such studies.

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