

The drug resistance-related protein LRP is the human major vault protein

GEORGE L. SCHEFFER¹, PETER L.J. WIJNGAARD³, MARCEL J. FLENS¹,
MIGUEL A. IZQUIERDO¹, MARILYN L. SLOVAK⁴, HERBERT M. PINEDO²,
CHRIS J.L.M. MEIJER¹, HANS C. CLEVERS³ & RIK J. SCHEPER¹

¹Department of Pathology and ²Department of Oncology, Free University Hospital, de Boelelaan 1117,
1081 HV Amsterdam, The Netherlands

³Department of Immunology, Academic University Hospital, Heidelberglaan 100,
3584 CX, Utrecht, The Netherlands

⁴Department of Cytogenetics, City of Hope National Medical Center, 1500 East Duarte Road,
Duarte, California 91010-0269, USA

Correspondence should be addressed to R.J.S.

Multidrug-resistant cancer cells frequently overexpress the 110-kD LRP protein (originally named Lung Resistance-related Protein). LRP overexpression has been found to predict a poor response to chemotherapy in acute myeloid leukaemia and ovarian carcinoma. We describe the cloning and chromosome localization of the gene coding for this novel protein. The deduced LRP amino acid sequence shows 87.7% identity with the 104-kD rat major vault protein. Vaults are multi-subunit structures that may be involved in nucleo-cytoplasmic transport. The *LRP* gene is located on chromosome 16, close to the genes coding for multidrug resistance-associated protein and protein kinase C- β , and may mediate drug resistance, perhaps via a transport process.

The effectiveness of chemotherapy is often hampered by intrinsic or acquired drug resistance of the tumour cells. Most common malignancies already show a poor response to chemotherapy during initial treatment. Some initially respond favourably to chemotherapy, but subsequently develop multidrug resistance¹. Transmembrane transporter molecules, notably P-glycoprotein (Pgp)² or the multidrug resistance-associated protein (MRP)^{3,4}, can mediate such drug resistance by acting as cytotoxic drug efflux pumps. Even so, multidrug-resistant (MDR) tumour cell lines have been described without overexpression of either Pgp or MRP (refs 5, 6, 7), indicating that other mechanisms may be operative.

We reported recently on the production of the mouse monoclonal antibody LRP-56, which detects a 110-kD protein that we have named Lung Resistance-related Protein (LRP), because it was first identified in an MDR lung cancer cell line⁸. LRP has been found to be overexpressed in all non-Pgp MDR tumour cell lines examined thus far, including the SW1573/2R120 (lung cancer), MCF-7/MITOX (breast cancer), HT1080/DR4 (fibrosarcoma) and 8226/MR40 (myeloma) cell lines⁸. Further support for a close association of LRP with a resistant phenotype was obtained by the observation that reversal of drug resistance in the SW1573/2R120 tumour cell line was accompanied by a subsequent decrease in expression of the protein⁸. Moreover, LRP expression in acute myeloid leukaemia⁹ and in advanced ovarian carcinoma¹⁰ was found to have a high predictive value for poor response to chemotherapy and adverse clinical outcome.

Molecular characterization of this novel gene is of crucial importance for further investigations into its role and the mechanism of action of its product in normal tissues and in

multidrug resistance. To this end, a eukaryotic expression system was used to isolate a full-length complementary DNA (cDNA) clone encoding LRP. The cDNA was sequenced and scanned for homology with other sequences. Isotope-labelled RNA probes were generated to detect the levels of the cognate RNA in MDR cell lines, and the chromosome localization of *LRP* was revealed by fluorescence *in situ* hybridization (FISH).

cDNA isolation and protein precipitation

The cDNA coding for the *LRP* gene product was isolated by expression cloning using mouse MOP8 cells and the LRP-56 monoclonal antibody to screen a cDNA library derived from the human non-Pgp multidrug-resistant fibrosarcoma cell line HT1080/DR4. Purified pCDM8 plasmids containing cDNA inserts were transfected into MOP8 cells and octo-cytospin preparations were screened for transiently expressed protein with the LRP-56 monoclonal antibody. The colony containing the *LRP* cDNA insert was isolated by screening progressively smaller pools of bacterial colonies (Fig. 1). Confirmation of the isolation of full-length cDNA was obtained by ³⁵S-immunoprecipitation. From both control MDR tumour cells and *LRP*-transfected MOP8 cells, but not from MOP8 cells transfected with irrelevant plasmid, the predicted *M_r* 110K protein was precipitated (Fig. 2). No precipitated protein was observed when the LRP-56 monoclonal antibody was replaced by an irrelevant antibody (data not shown).

cDNA sequence and mRNA expression

The cDNA was digested by restriction enzymes and subcloned in pBluescript SKII+. Sequence analysis of the full-length insert

and subcloned fragments was performed to obtain the complete cDNA sequence. The sequence displays a single open reading frame of 2,688 base pairs coding for an 896-amino acid protein with a calculated M_r of 100K. A computer-assisted search of the *LRP* cDNA versus the GenBank library revealed a very high level of similarity with the sequences coding for the major vault proteins (MVP) from *Dictyostelium discoideum* and *Rattus norvegicus*^{11,12}. MVP accounts for more than 70% of the composition of rat vaults¹³. Alignment of the deduced protein sequences of LRP and MVP- α from the slime mould *D. discoideum*, consisting of 896 and 843 amino acids, respectively, showed that 57% of the amino acids were identical. Alignment of LRP and MVP from *R. norvegicus* (895 amino acids), showed that 87.7% of the amino acids (785) are identical (Fig. 3). Like MVP- α from *D. discoideum*, the LRP sequence lacks the additional ten amino acids at the amino terminus. This supports the view that in the rat the second methionine is used as the authentic translation start¹¹.

To study the correlation between LRP protein and messenger RNA expression, a 232-nucleotide *Apal* fragment of the *LRP* cDNA sequence was selected to produce an isotope-labelled probe for RNase protection assays. Cytoplasmic RNA of MDR cell lines was isolated and tested for *LRP* mRNA levels. *LRP* mRNA in the non-Pgp MDR tumour cell line SW1573/2R120 is approximately 8 and 4 times as high as the SW1573/2R160, a Pgp-positive MDR subline with low LRP expression, and

SW1573/2R120 partial revertant, respectively. *LRP* mRNA in the MDR subline GLC4-ADR is approximately 14 times as high as in the drug-sensitive parental cell line GLC4-S (Fig. 4).

The full-length *LRP* cDNA was cloned into the eukaryotic expression vector pRc/CMV. Stable LRP-transfectants of the drug-sensitive A2780 ovarian carcinoma cell line that expressed significant levels of the LRP protein were obtained. These transfectants did not show increased resistance to doxorubicin, vincristine or VP16 as compared with untransfected A2780 cells (data not shown).

FISH localization

The complete *LRP* cDNA insert was biotin labelled for fluorescence *in situ* hybridization to normal human metaphases. Using a reference probe for the 16qter telomeric region (Oncor, P-5432), the *LRP* gene was found to be localized to the short arm of chromosome 16, within the 16p13.1–16p11.2 chromosomal region (Fig. 5).

Discussion

Major vault protein structure

The almost complete identity of the amino acid sequence of the human LRP protein and the rat major vault protein proves conclusively that LRP is the human major vault protein. Vaults were discovered by Kedersha and Rome in 1986 when electron microscopy showed small ovoid bodies in rat liver vesicle preparations^{13,14}. The cellular organelles are ribonucleoprotein particles containing four proteins of M_r 210K, 192K, 104K and 54K, respectively, and a species of small RNA of ~140 bases in the relative molar ratios of 3:1:55*:7:9 (*MVP: the LRP homologue). The particles measure approximately 35 × 65 nm, have a total mass of 13 MD, and are shaped as hollow, barrel-like structures with 822 symmetry, being composed of two identical cup-like halves joined at their open ends¹⁴.

Because vaults have been found in cells from amoebas to humans and have apparently been very well preserved throughout evolution, they are expected to play a key role in fundamental cell processes^{14,15}. Although a precise tissue distribution of vaults is not available, vaults have been reported to be most abundant in epithelial cells and macrophages¹⁵. We found LRP to be present in most normal human tissues, and highest expression was observed in epithelial cells with secretory and excretory functions, as well as in cells chronically exposed to xenobiotics, such as bronchial cells and cells lining the intestine⁸.

LRP expression

LRP overexpression was noted in a broad panel of human MDR tumour cell lines of different histogenetic origins⁸. Recently, in a panel of 61 drug-unselected human cancer cell lines¹⁶, we found LRP expression, rather than Pgp or MRP expression, to correlate with *in vitro* resistance to a range of anticancer drugs (M.A.I. *et al.*, manuscript submitted). Clinically, LRP expression, but not Pgp or MRP expression, was found to correlate with poor response to chemotherapy and adverse clinical outcome in acute myeloid leukaemia⁹ and in advanced ovarian carcinoma¹⁰. Taken together, the data obtained thus far point to a close association between LRP/vaults and chemotherapy resistance.

LRP chromosome 16 locus

The localization of the *LRP* gene on the short arm of chromosome 16 is of particular interest because this chromosomal re-

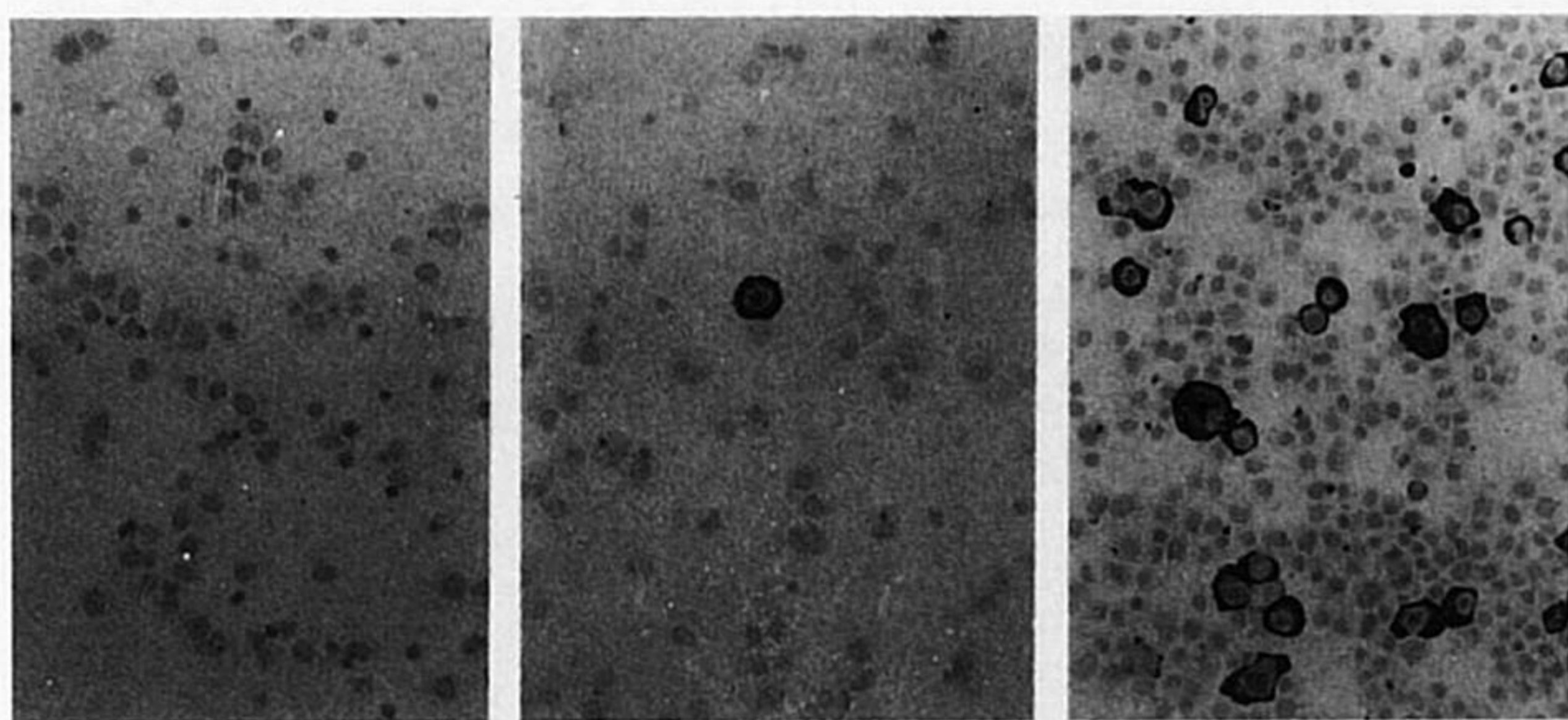
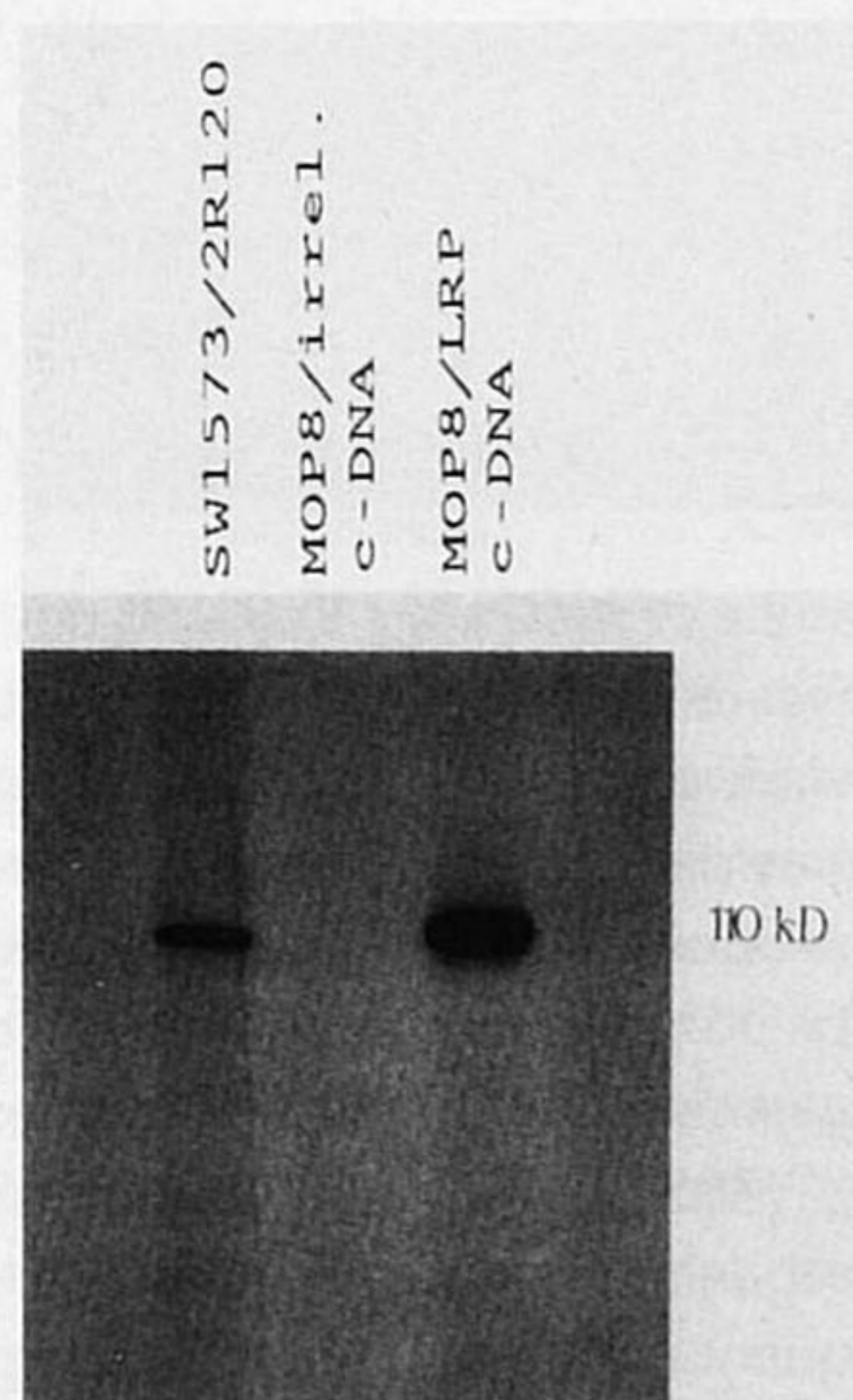


Fig. 1 Transiently transfected MOP8 cells, immunostained with LRP-56 monoclonal antibody. *a*, MOP8 cells transfected with irrelevant plasmids. *b*, Primary screening of the cDNA library in which a single LRP-positive cell was detected among 30×10^6 cells. *c*, Transfection with purified *LRP*-containing plasmid. Positive cells were revealed by 0.02% 9-amino-3-ethyl-carbazole (AEC)/0.02% hydrogen peroxide in 0.1 M sodium acetate, pH 4.8.

Fig. 2 [³⁵S]Methionine precipitation with LRP-56 monoclonal antibody. Left lane, MDR cells SW1573/2R120. Middle lane, MOP8 cells transfected with irrelevant cDNA. Right lane, *LRP*-transfected MOP8 cells. The specific 110-kD band is detected by autoradiography. The autoradiograph shown is a 48-hour exposure at -70 °C.



gion seems to be critically involved in MDR. Two other MDR-associated genes have been localized to this region: the MDR transporter MRP (16p13.1, ref. 17) and protein kinase C- β (16p11.2, ref. 18), a protein that augments transmembrane transport of drugs by phosphorylation of Pgp¹⁹.

Acute myeloid leukaemic patients with a deletion of one of the MRP genes, resulting from a chromosome 16 inversion, were reported to have a favourable outcome²⁰. We are currently investigating such patients for the presence of a further LRP deletion, which could contribute to or even account for this favourable outcome, as suggested by the prognostic value of LRP expression in patients with acute myeloid leukaemia.

The above-mentioned studies in leukaemia⁹, ovarian carcinoma¹⁰ and in the unselected human cancer cell lines provided evidence that MRP and LRP expression are not co-regulated. Although LRP and MRP were frequently co-expressed, many specimens showed high expression of only one of the two molecules, with low or no expression of the other. Apparently, the two genes, though located close to each other, can be switched on individually and relate independently to the MDR phenotype.

Major vault protein function

The function of vaults is still far from clear. Approximately 5% of the vaults are nucleus associated and localize to the nuclear pore complexes²¹. Nuclear pore complexes are multicomponent structures that allow a bidirectional nucleo-cytoplasmic exchange of molecules and particles²². Vault proteins are thought to constitute the transporters or central plugs of the nuclear pore complexes, controlling this exchange^{14,21}. The majority of vaults, however, have a cytoplasmic localization and were originally isolated in association with vesicular structures¹³. The coarsely granular, cytoplasmic staining pattern observed with the LRP-56 monoclonal antibody also suggests a vesicular localization for LRP⁵. LRP and/or vaults may regulate nucleocytoplasmic as well as vesicular transport of different substrates, including cytotoxic drugs. In support of this view, the decreased nucleus/cytoplasm ratio of drugs and sequestration of drugs in exocytotic vesicles has been reported in LRP-overexpressing MDR cell lines^{23,24}.

To further elucidate the contribution of the *LRP* gene product and vaults to cell physiology and multidrug resistance, we have produced stable *LRP* gene transfectants. Initial results of drug sensitivity testing with these transfectants indicate that transfection with just the *LRP* gene itself is insufficient to confer a MDR phenotype. Considering that the assembled functional vault structure is a multi-subunit particle, consisting of LRP/MVP and at least three other proteins as well as an RNA moiety, other approaches need to be explored. We are currently investigating the possibility of disrupting the assembling of functional vaults with *LRP* hammerhead ribozymes and examining what effect this will have on multidrug resistance.

In conclusion, the sequence and the chromosome localization of the novel and highly conserved human *LRP* gene is described. The evolutionary preservation of this major vault protein gene predicts a prominent physiological function. Its frequent upregulation in MDR tumour cells together with its value in predicting *in vitro* and clinical response to chemotherapy support the view that LRP and/or vaults can, next to members of the transmembrane transporter family, contribute to cytostatic drug resistance.

Methods

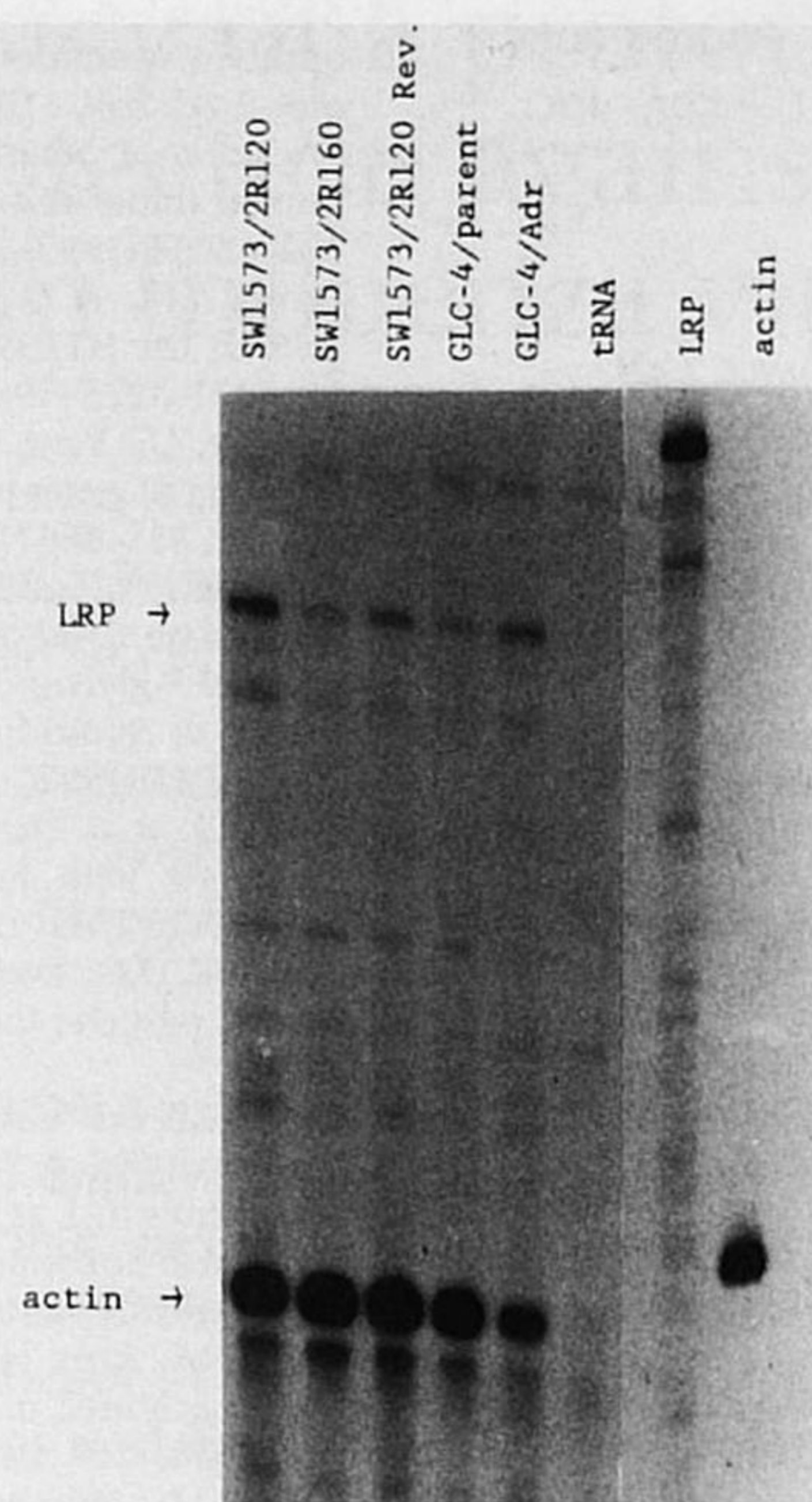
Cell lines. MDR sublines HT1080/Dr4 (ref. 25), GLC4-ADR²⁶ (kindly provided by E.G.E. de Vries, Groningen, Holland), SW1573/2R120 and SW1573/2R160 (ref. 27) (a Pgp-positive subline) were cultured in the presence of 185 nM, 1180 nM, 120 nM and 160 nM doxorubicin, respectively, once every week. Drug-sensitive A2780 ovarian carcinoma cells²⁸ and mouse 3T3 fibroblasts, MOP8 cells²⁹, were grown in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS) and antibiotics.

Preparation of cDNA sublibraries. The cDNA sublibraries were made as described^{30,31}. Poly(A)⁺ RNA was isolated from the non-Pgp MDR subline HT1080/DR4 using RNazol (Tell-Test, Inc., Friendswood, Texas)

LRP	MATEEFIIIRIPPHYIHLVDQNSNVSVEVGPQTYIRQDNERV	44
MVP <i>R. norv</i>	MFGGKSLGFTMATEEAIIRIPPHYIHLVDQNSNVSVEVGPQTYIRQDNERV	54
LRP	FAPRMVTVPPRHYCTVANPVSRAQGLVLFVDTGQVRLRHADLEIRLAQDPFP	98
MVP <i>R. norv</i>	FAPVRMVTVPVPRHYCIIVANPVSRTQSSVLFDTGQVRLRHADQEIIRLAQDPFP	108
LRP	LYPGEVLEKDIITPLQVLPNTALHLKALLDFEDKDGKVVAGDEWLFEGPGTYI	152
MVP <i>R. norv</i>	LYPGEVLEKDIITPLQVLPNTALHLKALLDFEDKNGDKVMAGDEWLFEGPGTYI	162
LRP	PRKEVEVVEIIQATIIIRQNQALRLRARKECWDRDGRVTEGEWLVTTVGAYLP	206
MVP <i>R. norv</i>	PQKEVEVVEIIQATVIKQNQALRLRARKECFDREGKGRVTEGEWLVRSVGYLP	216
LRP	AVFEEVLDLVDVAVILTEKTALHLRARRNFRDFRGVSRRTGEEWLVTVQDTEAHV	260
MVP <i>R. norv</i>	AVFEEVLDLVDVAVILTEKTALHLRALQNFRLRGLVHRTGEEWLVTVQDTEAHV	270
LRP	PDVHEEVLGVVPIITLGPVPHNYCVILDPVGPDPGKQLGQKRVVKGESFFLQPGGE	314
MVP <i>R. norv</i>	PDVYEEVLGVVPIITLGPVPHNYCVILDPVGPDPGKQLGQKRVVKGESFFLQPGGE	324
LRP	QLEQGIQDVYVLSSEQGLLLRALQPLEEGEDEEKVSHQAGDHWLIRGPLEYVPS	368
MVP <i>R. norv</i>	RLERGIQDVYVLSSEQGLLLRALQPLEEGESEKVSQAGDCWLIRGPLEYVPS	378
LRP	AKVEVVEERQAIPLDENEGIYVQDVKTGKVRVAVIGSTYMLTQDEVLWEKELPPG	422
MVP <i>R. norv</i>	AKVEVVEERQAIPLDQNEGIYVQDVKTGKVRVAVIGSTYMLTQDEVLWEKELPSG	432
LRP	VEELLNKGQDPLADRGEKDTAKSLQPLAPRNKTRVVSYPVPHNAAVQVYDYREK	476
MVP <i>R. norv</i>	VEELLNLGHDPLADRQKGTAKPLQPSAPRNKTRVVSYPVPHNAAVQVYDYRAK	486
LRP	RARVVFPELVSLGPEEQFTVLSLSAGRPKRPHARRALCLLLGPDFFTDVTITIE	530
MVP <i>R. norv</i>	RARVVFPELVSLDPEEQFTVLSLSAGRPKRPHARRALCLLLGPDFFTDVTITIE	540
LRP	TADHARLQLQLAYNWHFEVNDKDPQETAKLFSVPDFVGDACKAIASRVRGAVA	584
MVP <i>R. norv</i>	TADHARLQLQLAYNWHFELKRNNDPAEAAKLFVSPDFVGDACKAIASRVRGAVA	594
LRP	SVTFDDFHKNSARIIRTAVFVGFETSEAKGPDGMALPRPRDQAVFPQNLVSVSSV	638
MVP <i>R. norv</i>	SVTFDDFHKNSARIIRMAVFGFEMSEDTGPDGTLPLKARDQAVFPQNLVSVSSV	648
LRP	DVQSVPEVDQRTDALQRSVQLAIEITNSQEAQAQHEAQRLEQEARGLERQK	692
MVP <i>R. norv</i>	DVQSVPEVDQRTDALQRSVQLAIEITNSQEAQAQHEAQRLEQEARGLERQK	702
LRP	ILDQSEAEKARKELLELEALSMVESTGTAKAEASRAEAARIEGEGSVLQAKL	746
MVP <i>R. norv</i>	ILDQSEAEKARKELLELEALSMVESTGNAAEAASRAEAARIEGEGSVLQAKL	756
LRP	KAQALAIETEAELEQVQVRELELVYARAQLELEVSKAQQLAEVEVKKFKQMTTE	800
MVP <i>R. norv</i>	KAQALAIETEAELEQVQVRELELVYARAQLELEVSKAQQLANVEAKFKKEMTE	810
LRP	AIGPSTIRDLAVAGPEMQVLLQSLGLKSTLITDGTPIINLFNTAFGLLGMGPE	854
MVP <i>R. norv</i>	ALGPGTIRDLAVAGPEMQVLLQSLGLKSTLITDGTSSPMLNFSTASGCWGWGLM	864
LRP	GQPLGRRVPAQVWGGDIIPVCSGSPSSWRQPRGACTALTPD	896
MVP <i>R. norv</i>	VSRQHRSDPAAR-----GRLALPGSPKQPCCALGQH	895

Fig. 3 Deduced LRP amino acid sequence aligned with the major vault protein from *Rattus norvegicus*. The LRP amino acid sequence was deduced from sequence analysis of the full-length insert and of subcloned fragments. The alignment was generated with the PALIGN program of PC-gene with an open gap cost of 10 and a unit gap cost of 2. Identical and conserved amino acids are identified by '|' (785; 87.7%) and '.' (23; 2.6%), respectively. The *LRP* nucleotide sequence is available at the EMBL, GenBank and Nucleotide Sequence databases under accession number X79882.

Fig. 4 RNase protection assay analysis of *LRP* mRNA in MDR cell lines. Protected *LRP* probe is indicated by the upper arrow, the lower arrow indicates protected γ -actin probe as an RNA recovery control. *LRP* mRNA in the non-Pgp MDR tumour cell line SW1573/2R120 is approximately 8- and 4-fold as high as the SW1573/2R160, a Pgp-positive MDR subline with low *LRP* expression, and SW1573/2R120 partial revertant, respectively. *LRP* mRNA in the MDR cell line GLC4-ADR is approximately 14-fold as high as in the sensitive GLC4-S parental cell line. Intensities of protected RNA fragments were analysed on an LKB Ultrascan XL densitometer (Pharmacia AB, Bromma, Sweden). The autoradiograph shown is a 16-hour exposure at -70°C .



and an oligo dT column. A size-fractionated ($>2,000$ bp) oligo dT-primed cDNA library was constructed in a pCDM8 vector using non-self complementary *Bst*XI linkers. High-voltage electroporation ($25\ \mu\text{F}$, $200\ \text{ohm}$, $2.5\ \text{kV cm}^{-1}$) of the library into the *Escherichia coli* strain MC1061/p3 yielded approximately 100,000 primary colonies. These were divided into ten sublibraries of 10,000 colonies each.



Fig. 5 Fluorescence *in situ* hybridization (FISH) of *LRP* cDNA to a normal metaphase. FISH was performed using a 2.8-kb biotin-labelled *LRP* cDNA, detected with FITC-avidin (green) and a digoxigenin-labelled reference cosmid probe specific for the 16qter telomeric region (Oncor, P-5432), detected with rhodamine-anti-digoxigenin (red) according to the chromosomal *in situ* hybridization protocol for unique sequence probes. The chromosomes were counterstained with diamidine-phenylindole dihydrochloride (DAPI). *LRP* (arrow) is localized to the short arm of chromosome 16 within the 16p13.1-16p11.2 chromosomal region.

Isolation of the *LRP* cDNA clone. Trypsinized MOP8 cells (5×10^6 per sample) were transfected for 45 minutes at 37°C in 2 ml RPMI 1640 containing 2% Nu serum (Becton Dickinson, Bedford, Massachusetts), $250\ \mu\text{g ml}^{-1}$ DEAE-dextran and $1\ \mu\text{g ml}^{-1}$ of purified plasmid from the sublibraries. The cells were washed once and cultured in Dulbecco's modified Eagle's medium, containing 10% FCS and antibiotics for 48 hours. Cells were trypsinized, spun down onto octo-cytospin slides, dried, fixed in acetone for 5 minutes and immunostained for transiently expressed protein with LRP-56 mAb. Positive cells were seen with 0.02% 9-amino-3-ethyl-carbazole (AEC)/0.02% hydrogen peroxide in 0.1 M sodium acetate, pH 4.8. The colony containing the *LRP* cDNA was isolated from one of the sublibraries by screening progressively smaller pools of bacterial colonies.

cDNA sequencing. Restriction enzyme fragments of the *LRP* cDNA were purified and subcloned in pBluescript SKII+. dsDNA sequencing of the full-length insert and subcloned fragments was performed according to the dideoxynucleotide method³², by using T7 polymerase and ^{35}S -labelled

nucleotides. Bands were revealed by autoradiography.

Immunoprecipitation assay. MOP8 cells, 24 hours after transfection with *LRP* or irrelevant plasmids, and MDR control cells were used in the immunoprecipitation assay as previously described⁸. Cells were labelled overnight with $4\ \mu\text{Ci ml}^{-1}$ [^{35}S]methionine. Equal counts per minute (25×10^6 /sample) of clear supernatants were precipitated with $10\ \mu\text{g}$ of LRP-56 mAb or $10\ \mu\text{g}$ of irrelevant mAb. Precipitated protein was shown by autoradiography.

RNase protection assay. RNase protection assay was performed as described³³. Cytoplasmic RNA of the cell lines ($10\ \mu\text{g}$) was hybridized with α - ^{32}P -labelled RNA transcripts complementary to *LRP* mRNA sequences or γ -actin. The *LRP* probe was transcribed from *Eco*RI-linearized DNA from a *LRP*-*Apal* fragment (nucleotides 2,326-2,558) in pBluescript using T7 RNA polymerase. The DNA template was removed by DNase I treatment. After RNase A treatment, protected probe was visualized by electrophoresis through a denaturing 6% acrylamide gel, followed by autoradiography.

Stable transfectants and chemosensitivity testing. *LRP* full-length cDNA was cloned into the eukaryotic expression vector pRc/CMV (Invitrogen). A2780 cells were transfected with $5\ \mu\text{g}$ of purified plasmid by calcium phosphate precipitation⁴. Stable transfectants were selected for three weeks in medium containing $800\ \mu\text{g ml}^{-1}$ G418 (ref. 4). Transfectants were tested for expression of LRP protein by immunohistochemistry. Resistance of stable *LRP* transfectants to doxorubicin, vincristine and VP16 was determined by [^3H]thymidine incorporation after a 3-day culture period in different concentrations of drug. Untransfected A2780 cells were used as a control. SW1573/2R120 MDR cells were used as a positive control.

FISH. Full-length *LRP* cDNA was biotin-labelled by nick translation (Boehringer nick-translation kit). Slides containing metaphases were treated per manufacturer's instructions for the Oncor chromosome *in situ* hybridization system for single-copy sequence detection in metaphase chromosomes using fluorescein isothiocyanate-labelled avidin/anti-avidin antibody for amplification of the signal. A digoxigenin-labelled cosmid probe specific for the 16qter telomeric region (Oncor, P-5432), detected with rhodamine-anti-digoxigenin, was used as a reference probe. Metaphase analysis and photomicroscopy

were performed using a Nikon photomicroscope. Photographs were taken using the Oncor imaging system (Oncor, Inc., Gaithersburg, Maryland).

Acknowledgements

We thank M. v.d. Wetering and J.P. Ho for technical assistance, D.H. Joziase and G.J.R. Zaman for discussion, C. van Rijn for secretarial assistance, and J. Wagstaff for critical reading of the manuscript. This work was in part supported by a grant from Bristol-Myers Squibb Company.

RECEIVED 3 JANUARY; ACCEPTED 20 APRIL 1995

- Roninson, I.B. *Molecular and Cellular Biology of Multidrug Resistance in Tumor Cells* (Plenum, New York, 1991).
- Endicott, J.A. & Ling, V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu. Rev. Biochem.* **58**, 137-171 (1989).
- Cole, S.P.C. *et al.* Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* **258**, 1650-1654 (1992).
- Zaman, G.J.R. *et al.* The human multidrug resistance-associated protein MRP is a plasma membrane drug-efflux pump. *Proc. natn. Acad. Sci. U.S.A.* **91**, 8822-8826 (1994).
- Futscher, B.W., Abbaszadegan, M.R., Domann, F. & Dalton, W.S. Analysis of MRP mRNA in mitoxantrone-selected, multidrug-resistant human tumor cells. *Biochem. Pharmacol.* **47**, 1601-1606 (1994).
- Takeda, Y. *et al.* Reversal of multidrug resistance by tyrosine-kinase inhibitors in a non-P-glycoprotein-mediated multidrug-resistant cell line. *Int. J. Cancer* **57**, 229-239 (1994).
- Slapak, C.A. *et al.* Overexpression of the multidrug resistance-associated protein (MRP) gene in vincristine but not doxorubicin selected multidrug-resistant murine erythroleukemia cells. *Cancer Res.* **54**, 5607-5613 (1994).
- Scheper, R.J. *et al.* Overexpression of a M(r) 110,000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance. *Cancer Res.* **53**, 1475-1479 (1993).
- List, A.F. *et al.* Non-P-glycoprotein (Pgp) mediated multidrug resistance (MDR): Identification of a novel drug resistance phenotype with prognostic relevance in acute myeloid leukemia (AML). *Blood* **82**, (suppl. 1) 443a (1993).
- Izquierdo, M.A. *et al.* Prognostic significance of the drug resistance associated protein LRP in advanced ovarian carcinoma. *Ann. Oncol.* **5**, (suppl. 8), 98 (1994).
- Kickhoefer, V.A. & Rome, L.H. The sequence of a cDNA encoding the major vault protein from *Rattus norvegicus*. *Gene* **151**, 257-260 (1994).
- Vasu, S.K., Kedersha, N.L. & Rome, L.H. cDNA cloning and disruption of the major vault protein alpha gene (*mvpA*) in *Dictyostelium discoideum*. *J. Biol. Chem.* **268**, 15356-15360 (1993).
- Kedersha, N.L. & Rome, L.H. Isolation and characterization of a novel ribonucleoprotein particle: Large structures contain a single species of small RNA. *J. Cell Biol.* **103**, 699-709 (1986).
- Rome, L.H., Kedersha, N.L. & Chugani, D.C. Unlocking vaults: Organelles in search of a function. *Trends Cell Biol.* **1**, 47 (1991).
- Kedersha, N.L., Miquel, M.C., Bittner, D. & Rome, L.H. Vaults II. Ribonucleoprotein structures are highly conserved among higher and lower eukaryotes. *J. Cell Biol.* **110**, 895-901 (1990).
- Wu, L. *et al.* Multidrug-resistant phenotype of disease-oriented panels of human tumor cell lines used for anticancer drug screening. *Cancer Res.* **52**, 3029-3034 (1992).
- Slovak, M.L. *et al.* Localization of a novel multidrug resistance-associated gene in the HT1080/DR4 and H69AR human tumor cell lines. *Cancer Res.* **53**, 3221-3225 (1993).
- Francke, U., Yang Feng, T.L., Brissenden, J.E. & Ullrich, A. Chromosomal mapping of genes involved in growth control. *Cold Spring Harb. Symp. quant. Biol.* **51**, 855-866 (1986).
- Chambers, T.C., Zheng, B. & Kuo, J.F. Regulation by phorbol ester and protein kinase C inhibitors, and by a protein phosphatase inhibitor (okadaic acid), of P-glycoprotein phosphorylation and relationship to drug accumulation in multidrug-resistant human KB cells. *Molec. Pharmacol.* **41**, 1008-1015 (1992).
- Kuss, B.J. *et al.* Deletion of gene for multidrug resistance in acute myeloid leukaemia with inversion in chromosome 16: Prognostic implications. *Lancet* **343**, 1531-1534 (1994).
- Chugani, D.C., Rome, L.H. & Kedersha, N.L. Evidence that vault ribonucleoprotein particles localize to the nuclear pore complex. *J. Cell Sci.* **106**, 23-29 (1993).
- Rout, M.P. & Wente S.R. Pores for thought: Nuclear pore complex proteins. *Trends Cell Biol.* **4**, 357-365 (1994).
- Schuurhuis, G.J. *et al.* Early multidrug resistance, defined by changes in intracellular doxorubicin distribution, independent of P-glycoprotein. *Br. J. Cancer* **64**, 857-861 (1991).
- Dietel, M., Arps, H., Lage, H. & Niendorf, A. Membrane vesicle formation due to acquired mitoxantrone resistance in human gastric carcinoma cell line EPG85-257. *Cancer Res.* **50**, 6100-6106 (1990).
- Slovak, M.L., Hoeltge, G.A., Dalton, W.S. & Trent, J.M. Pharmacological and biological evidence for differing mechanisms of doxorubicin resistance in two human tumor cell lines. *Cancer Res.* **48**, 2793-2797 (1988).
- Zijlstra, J.G., de Vries, E.G.E. & Mulder, N.H. Multifactorial drug resistance in an Adriamycin-resistant human small-cell lung carcinoma cell line. *Cancer Res.* **47**, 1780-1784 (1987).
- Kuiper, C.M. *et al.* Drug transport variants without P-glycoprotein overexpression from a human squamous lung cancer cell line after selection with doxorubicin. *J. Cell Pharm.* **1**, 35-41 (1990).
- Eva, A. *et al.* Cellular genes analogous to retroviral *onc* genes are transcribed in human tumour cells. *Nature* **295**, 116 (1982).
- Muller, W.J., Naujokas, M.A. & Hassell, J.A. Isolation of large T antigen-producing cell lines capable of supporting replication of polyomavirus-plasmid recombinants. *Molec. Cell Biol.* **4**, 2406-2412 (1984).
- Wijngaard, P.L.J., Metzelaar, M.J., McHugh, N.D., Morrison, W.I. & Clevers, H.C. Molecular characterization of the WC1 antigen expressed specifically on bovine CD4- CD8- gamma delta T lymphocytes. *J. Immun.* **149**, 3273-3277 (1992).
- Aruffo, A. & Seed, B. Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. *Proc. natn. Acad. Sci. U.S.A.* **84**, 8573-8577 (1987).
- Sanger, F., Nicklen, S. & Coulson, A.R. DNA sequencing with chain-terminating inhibitors. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
- Zinn, K., Dimaio, D. & Maniatis, T. Identification of two distinct regulatory regions adjacent to the human beta-interferon gene. *Cell* **34**, 865-879 (1983).