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


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 resistance.1 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 cytoplasmic 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 line.1 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 (melanoma) cell lines.5 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 protein.6 Moreover, LRP expression in acute myeloid leukaemia and in advanced ovarian carcinoma 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 where transfected into MOP8 cells and octo-cytosin 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, 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