Vault-related resistance to anticancer drugs determined by the expression of the major vault protein LRP

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Abstract

In this review we analyze the data supporting the notion that vault-related MDR, as reflected by LRP/MVP overexpression, represents a marker of drug resistance *in vitro* and in the clinic. Vaults, besides playing a fundamental biological role, may be involved in a novel mechanism of MDR.

Abbreviations: ABC – ATP-binding cassette; LRP – Lung Resistance-related Protein; MVP – Major Vault Protein; MDR – Multidrug Resistance; MRP – Multidrug Resistance Protein; NPC – Nuclear Pore Complex; Pgp – P-glycoprotein

Introduction

Broad resistance to currently available chemotherapeutic drugs is a major cause of failure of cancer treatment. Extensive laboratory studies on the mechanisms of drug resistance have focused on drug-selected resistant cancer cell lines in which a myriad of genetic, molecular and biochemical changes have been observed as compared to the parental cell lines.

One type of *in vitro* drug resistance termed multidrug resistance (MDR) is characterized by resistance to a variety of structurally and functionally diverse drugs including anthracyclines, epipodophyllotoxins, *vinca* alkaloids and taxanes (Biedler, 1994).

In experimental systems, MDR is known to be conferred by two proteins, the *MDR1* gene product, P-glycoprotein (Pgp), and the *MRP* gene product, Multidrug Resistance Protein (MRP). These two proteins belong to the ATP-binding cassette (ABC) superfamily of transporter proteins (Higgins, 1992). The mechanism(s) by which Pgp mediates drug resistance, as well as its possible physiological function

and clinical relevance have been reviewed extensively by others (Goldstein, 1992). The mechanism(s) by which MRP mediates drug resistance and its clinical relevance are beginning to be elucidated (Loe, 1996). Besides ABC transporters, other mechanisms may exist that contribute to diverting cytostatic drugs from their intracellular targets, thereby conferring MDR. We discovered a protein overexpressed in a MDR lung cancer cell line, termed Lung Resistance-related Protein (LRP), which appeared to be a good candidate (Scheper, 1993). Subsequently LRP was identified as the human major vault protein (MVP), the predominant component of recently discovered cellular organelles named vaults (Scheffer, 1995). In this review we analyze the data supporting the notion that vault-related MDR, as reflected by LRP/MVP overexpression, represents a marker of drug resistance *in vitro* and in the clinic. Vaults, besides playing a fundamental biological role, may be involved in a novel mechanism of MDR.

Discovery and Molecular Characterization of LRP

Discovery of LRP

Production of the monoclonal antibody LRP-56

The SW-1573/2R120 MDR cell line was derived from SW-1573 non-small cell lung cancer cells by exposure to increasing concentrations of doxorubicin. The 2R120 subline is characterized by moderate levels of cross-resistance to doxorubicin, vincristine, and etoposide (4- to 45-fold), reduced intracellular drug accumulation, and absence of *MDR1*/Pgp expression (Kuiper, 1990). BALB/c mice were immunized with 2R120 cells and the monoclonal antibody LRP-56 was selected for strong immunoreactivity with 2R120 cells as compared to parental SW-1573 cells (Scheper, 1993). In the 2R120-revertant cell line (2R120 cells cultured without drug for over nine months), which shows a decrease in the level of resistance similar to parental SW-1573 cells, a decrease in the level of LRP expression was also observed, further supporting a close association between LRP and drug resistance in 2R120 cells (Scheper, 1993).

Pattern of LRP-56 staining

LRP-56 displayed a characteristic cytoplasmic punctate staining pattern in the 2R120 cells (Scheper, 1993). The same cytoplasmic staining pattern has been subsequently observed in all other LRP-56-positive MDR cell lines tested, in drug-unselected cancer cell lines, in normal human tissues, and in human malignancies (see below) (Izquierdo, 1995; Izquierdo, 1996a, 1996b; Scheffer, 1995).

LRP-56 detects the 110 kDa protein, LRP

Immunoprecipitation studies showed that LRP-56 specifically reacted with a protein of approximately 110 kDa. This protein was named the Lung Resistance-related Protein (LRP) (Scheper, 1993). By immunocytochemistry and immunoprecipitation, LRP was initially found to be overexpressed in various Pgp-negative MDR cell lines, including the fibrosarcoma cell line HT1080/DR4, the small cell lung cancer cell line GLC4/ADR, and the myeloma cell line 8226/MR40 (Scheper, 1993). The prospect of a newly identified protein related to MDR stimulated studies to characterize LRP at the molecular level, as well as to establish its relationship with drug resistance in other *in vitro* models and in clinical tumour specimens.

Molecular characterization of LRP as the human major vault protein (MVP)

Cloning and sequencing of the LRP gene

The cDNA coding for the LRP gene product was isolated by expression cloning using mouse MOP8 cells and LRP-56 to screen a cDNA library generated from the MDR human fibrosarcoma cell line HT1080/DR4 (Scheffer, 1995). From both control MDR tumour cells and LRP-transfected MOP8 cells, but not from MOP8 cells transfected with irrelevant plasmid, the predicted 110 kDa protein was precipitated, thus confirming the isolation of full-length cDNA. The LRP sequence was found to display a single open reading frame of 2688 basepairs coding for an 896-amino-acid protein with a calculated Mr of 100 kDa. Several potential phosphorylation motifs for protein kinase C, casein kinase II, and tyrosine-protein kinase, as well as an amidation site, a phosphopantetheine attachment site and an EF-hand calcium binding domain were identified. Evidence has been shown that the MVP localized in nerve terminals of *Torpedo marmorata* becomes phosphorylated *in vitro* and *in vivo* (Herrmann, 1996). No indication of transmembrane fragments or the ATP binding 'active transport' signature that is characteristic for the transmembrane transporter proteins Pgp and MRP were present.

Chromosome location of the LRP gene

Using fluorescence *in situ* hybridization, the LRP gene has been localized to the short arm of chromosome 16, within the 16p11.2-16p13.1 chromosomal region, close to the MRP-gene site (Scheffer, 1995; Slovak, 1995). The protein kinase C- β gene, involved in MDR by activating the *MDR1* gene, and possibly the LRP gene as well (Komarov, 1997), has also been mapped to this region.

LRP is the human major vault protein (MVP)

LRP showed strong homology with the MVP from *Dictyostelium discoideum* and *Rattus norvegicus* (Kickhoefer, 1994; Scheffer, 1995; Vasu, 1995). Alignment of the protein sequences of LRP and rat MVP showed that 87.7% of the amino acids are identical. The strong identity found between the sequences of the human LRP protein and the rat MVP makes it evident that LRP is the human MVP (Scheffer, 1995). The MVP is the predominant component of recently described cellular organelles named vaults (Rome, 1991).

Vaults: Cellular Organelles in Search of a Function

Vaults composition and structure

Rome *et al.* described in 1986 hitherto unknown cell organelles which they named vaults, a term chosen to describe the morphology of the particles consisting of multiple arches reminiscent of those that form cathedral vaults (Kedersha, 1986). Vaults were first identified by negative staining and transmission electron microscopy as contaminant particles of clathrin-coated vesicle preparations derived from rat liver (Kedersha, 1986; Rome, 1991). Vaults are ribonucleoprotein particles which are composed of a MVP of 104 kDa (accounting for > 70% of the mass of the particle), three minor proteins of 210, 192, and 54 kD, and a small RNA molecule. The vault components are assembled in a barrel-like structure of approximately 57X32 nm with a molecular mass of about 13 MDa, composing the largest ribonucleoprotein body reported to date (three times the size of a ribosome). The vault particle has 2-fold symmetry and each half can be opened into a flower-like structure which contains eight petals surrounding a central ring (Kedersha, 1991). These dynamic structural variations are likely to play a role in vault function.

Evolutionary conservation of vaults

Vaults have been isolated from various species including the lower eukaryote *D. discoideum*, amphibians (frog), avians (chicken), and mammals (rat, rabbit, cow) (Kedersha, 1990; Rome, 1991). Vaults are most abundant in epithelial cells (i.e. rat intestine) and macrophages (i.e. rabbit alveolar macrophages) (Kedersha, 1990). As noted before, the amino acid composition of the MVP is highly conserved through evolution: the identity between the mammalian MVPs is approximately 90% (Herrmann, 1996; Scheffer, 1995). The potential phosphorylation sites are evolutionary conserved in all MVPs (Herrmann, 1996; Scheffer, 1995). Antibodies raised against rat vaults recognize the MVP in all eukaryotic species tested, including *Drosophila*, dog, and human. Furthermore, despite the complex composition and structure of vaults, these are highly conserved among phylogenetically dissimilar species, supporting the notion that their function is essential to eukaryotic cells (Kedersha, 1990; Kedersha, 1991). The size of the vault mRNA varies among species (Kickhoefer, 1993). By

cloning the human gene for vRNA and careful quantitation of vRNA levels in MDR cells the presence of a general pool of vRNA was demonstrated from which a certain fraction is associated with the vault particle at any time (Kickhoefer, 1998).

Cellular location of vaults

The majority of vaults is present in the cytoplasm and most cells contain thousands of vaults. A small fraction of vaults are localized to the nuclear membrane and nuclear pore complexes (NPC) (Chugani, 1993). Here structural similarities support the hypothesis that vaults constitute the central plugs of the NPC. Vaults have also been localized abundantly in cholinergic nerve terminals in close proximity to synaptic vesicles (Herrmann, 1996).

Function of vaults

The precise function of vaults is unknown. The location of vaults at the NPC and the data suggesting that vaults constitute, indeed, the transporter units of NPC raises the possibility that vaults mediate the bidirectional transport of a variety of substrates between the nucleus and the cytoplasm (Chugani, 1993; Rome, 1991). The association of cytoplasmic vaults with clathrin-coated vesicles from rat liver and with synaptic vesicles in nerve terminals in *Torpedo* raises the possibility that vaults may be implicated in vesicular transport processes (Herrmann, 1996; Kedersha, 1986). However, at present it is not clear whether the apparent colocalization of vaults with vesicles is due to a real physical link. Vault subpopulations have also been observed at the ruffling edges of spreading rat fibroblasts and along cytoskeletal elements, suggesting a possible role in motility (Rome, 1991). In the developing rat brain vaults are highly enriched in microglia (Chugani, 1991). Mutational analysis of MVP in slime mold indicates an involvement of vaults in cell growth (Vasu, 1995). The data obtained so far suggest that vaults play a central role in cell homeostasis (Herrmann, 1996; Rome, 1991).

Association of LRP/MVP with Drug Resistance

Frequent overexpression of LRP/MVP in drug-selected and drug-unselected cancer cell lines

LRP/MVP in drug-selected cancer cell lines

Overexpression of LRP/MVP has been found in a

large number of drug-selected MDR cell lines of various histogenetic origins and selected by different drugs (Moran, 1997; Scheper, 1993; Verovski, 1996), indicating that diverse cancer cells react by up-regulating the expression of the *LRP* gene after exposure to anti-cancer agents. Overexpression of LRP/MVP is not only seen in highly resistant MDR cell lines, but it is also frequently observed at the early steps of resistance selection (Moran 1997; Verovski, 1996; Versantvoort, 1995; Wyler, 1997). The mechanism(s) of drug resistance operative in these LRP/MVP-overexpressing MDR sublines displaying low levels of drug resistance are probably more clinically relevant, pointing to LRP/MVP as a potential useful marker of clinical resistance.

LRP/MVP in drug unselected cancer cell lines

Laboratory selected MDR cancer cells generated through stringent drug-treatment selection procedures show, in general, (very) high levels of resistance, making it difficult to extrapolate the results obtained to the clinic. Cancer cell lines not subjected to laboratory drug selection may reflect more accurately the biology, including chemoresistance pathways, of the tumours from which they have been derived. LRP/MVP was found to be expressed at various levels in 78% of 61 human cancer cell lines of different histogenetic origins used at the National Cancer Institute (NCI; USA) for screening of new anticancer drugs supporting that the LRP/MVP-associated mechanism of resistance is widespread in human malignancies (Izquierdo, 1996b). Clinical studies have confirmed the high frequency of LRP/MVP expression in cancer specimens (see section 5.2 and 6).

LRP/MVP expression related to Pgp and MRP expression

Intriguing relationships between the expression of LRP/MVP and Pgp or MRP in cancer cell lines have been observed. In drug-selected MDR cell lines expression of LRP/MVP and Pgp appears to be exclusive, since most Pgp-overexpressing MDR cell lines are LRP/MVP negative and *vice versa* (Moran, 1997; Scheper, 1993). In series of MDR cell lines up-regulation of LRP/MVP at low level of resistance with switching to up-regulation of Pgp at high levels of resistance is a frequent observation (Moran, 1997; Scheper, 1993; Versantvoort, 1995). This finding points to LRP/MVP, as opposite to Pgp, as a marker of low levels of drug resistance which probably are

more clinically relevant. Concomitant overexpression of LRP/MVP and Pgp has been found in few MDR cell lines, such as MCF7/D40 breast cancer cells, OAW42-SR ovarian carcinoma cells, and certain 8226 myeloma sublines (Moran, 1997; Scheper, 1997). Although most drug-unselected cancer cell lines from the NCI panel expressed LRP/MVP but not Pgp, co-expression of both MDR-related proteins was also seen (Izquierdo 1996b). In these LRP/MVP- and Pgp-positive cell lines, the LRP/MVP-associated mechanism of MDR may contribute to the MDR phenotype. This is supported by the different levels of resistance in two 8226 MDR sublines with similar Pgp content, but distinct LRP/MVP levels (higher resistance in the LRP/MVP positive subline) (Shao, 1995) or the high MDR phenotype in LRP/Pgp positive cell lines from the NCI panel (Izquierdo, 1996b). In certain cell lines, but not in all, protein kinase C appears to be a common mediator for the activation of the *MDR1* and *LRP* genes (Komarov, 1997).

In contrast to Pgp, most LRP/MVP overexpressing MDR cell lines display increased levels of MRP as well (Flens, 1994; Scheper, 1993). The concomitant operation of several drug resistance mechanisms may be often necessary to cause the phenotype of drug resistance observed in LRP/MVP and MRP positive drug-selected MDR tumour cells. In the NCI panel, co-expression of LRP/MVP and MRP was also frequent and, in general, associated with higher drug resistance levels (Izquierdo, 1996b). Despite the frequent co-upregulation and close chromosomal localization of the *LRP* and *MRP* genes (see above), evidence indicates that they do not belong to the same amplicon and can be switched on separately.

Amplification of the *MRP* gene, but not of the *LRP* gene, has been found in H69AR and GLC4/ADR small cell lung cancer MDR cells, and HL-60/AR leukaemia MDR cells (Slovak, 1995). Indeed, amplification of the *LRP* gene in LRP/MVP overexpressing MDR cell lines seems to be a rare event; to date only the HT1080/DR4 fibrosarcoma MDR cell line has been shown to display *LRP* gene amplification (Laurencot, 1997; Slovak, 1995).

In HCT15 and HCT116 colon carcinoma cells, tumour necrosis factor- α reduced *LRP* gene expression both at mRNA and protein level, similarly to its effect on the *MDR1* gene, whereas it increased *MRP* gene expression (Stein, 1997). Additional data indicate that the genes coding for Pgp, MRP and LRP/MVP are differentially regulated by 12-*O*-tetradecanolyphorbol-13-acetate, and cytotoxic drugs (Komarov, 1997).

Furthermore, in drug-unselected cancer cell lines and clinical tumour specimens, expression of only one of these proteins is not uncommon (Izquierdo, 1995; Izquierdo, 1996b).

Remarkably, LRP/MVP overexpression has also been reported in Pgp/MRP negative MDR cell lines, such as the mitoxantrone selected MCF7/MR cell line (Futscher, 1994). In these cells, the LRP/MVP-associated mechanism of MDR may play a prominent role.

The LRP/MVP-associated phenotype of drug resistance in vitro

As noted previously, overexpression of LRP/MVP is a frequent reaction of cancer cells of different histogenetic origins after exposure to various cytotoxic agents. In general, increased levels of LRP/MVP have been consistently associated with increasing levels of drug resistance and the reduction of resistance observed in revertant variants is paralleled by a decrease in LRP/MVP content (Scheper, 1993). The range of drugs used for the selection of LRP/MVP overexpressing drug resistant sublines is broad, including doxorubicin, mitoxantrone, etoposide, vincristine, cytarabine, methotrexate, and cisplatin (Ikeda, 1997; Komarov, 1997; Moran, 1997; Parker, 1997; Scheper 1993; Wyler, 1997). Thus, overexpression of LRP/MVP is not only associated with classical MDR drugs, but also with drugs not included in the classical MDR phenotype. Most MDR cell lines with LRP/MVP overexpression have been selected with doxorubicin, but show cross-resistance to etoposide, vincristine and other MDR-related drugs (Moran, 1997; Scheper, 1993). In A2780/CVP70 ovarian carcinoma cells, which have been selected for resistance to cisplatin, LRP/MVP overexpression was associated with resistance to this drug (Parker 1997). On the other hand, selection for resistance to cisplatin does not always result in overexpression of LRP/MVP.

The association of LRP/MVP with a broad spectrum of drug resistance is not limited to cancer cell lines selected for resistance in the laboratory. The relation of LRP/MVP, as well as of Pgp and MRP, to drug resistance was studied in the NCI panel of 61 human cancer cell lines (Izquierdo, 1996b). Using immunocytochemistry, LRP/MVP and MRP were found to be expressed in 78 and 87% of the cell lines, respectively, whereas Pgp was detected at relatively low levels in 24% of the cell lines. Among the three MDR-associated proteins, LRP/MVP showed the greatest

individual value as a marker of *in vitro* resistance to both MDR related drugs (i.e. doxorubicin, vincristine) and also to non-classical MDR drugs (i.e. cisplatin, carboplatin and melphalan) (Izquierdo, 1996b). In 8 of these cell lines, *LRP* mRNA expression, but not *MRP* mRNA expression, was determined to be strongly correlated with drug sensitivity to both MDR and non-MDR-related drugs. In these cell lines, *LRP* mRNA expression was somewhat better indicator of drug sensitivity than LRP/MVP protein expression (Laurencot, 1997). In another series of gastric cancer cell lines, a correlation was found between *LRP* mRNA levels and resistance to etoposide and vincristine.

Interestingly, in both gastric and lung cancer cell lines a correlation with resistance to SN-38, a DNA topoisomerase inhibitor, was observed (Ikeda, 1997). Although overexpression of LRP/MVP is, in general, paralleled by an increase in drug resistance, in OAW42-S ovarian carcinoma cells an augment in LRP/MVP expression in later passages of these cells did not result in an increase in drug resistance suggesting the presence of a non-functional form of LRP/MVP (Moran, 1997).

The association of the expression of LRP/MVP with a broad phenotype of *in vitro* drug resistance, including anticancer agents from various sources and mechanisms of action, has been confirmed in clinicopathological studies (see below).

The whole vault particles are up-regulated in MDR cancer cells

Kickhoefer *et al.* (1997) have shown that in LRP/MVP-overexpressing MDR cancer cells the up-regulation of the *LRP* gene is accompanied by up to a 15-fold increase in the synthesis of the whole vault particles. The formation of vaults seems to be limited by the expression of the major vault protein LRP or the other minor vault proteins, but not by the synthesis of vault RNA which is in excess to LRP/MVP. The fact that cancer cells exposed to cytotoxic anticancer agents respond synthesising such complex particles as vaults supports a direct role for vaults in drug resistance.

Expression of LRP/MVP in Normal Human Tissues and Primary Tumour Panels

LRP/MVP expression in normal tissues

Proteins related to drug resistance *in vitro* have been

demonstrated in various normal human tissues where they may play a protective role against toxic compounds and their malignant counterparts where the cells may retain the protective function neutralizing the cytotoxic effects of anticancer drugs. Vaults, as reflected by the expression of LRP/MVP, have been found to be widely distributed in human normal tissues and tumours (Izquierdo, 1996a; Sugawara, 1997). However, distinct patterns of expression were noticed. High LRP/MVP expression was seen in tissues chronically exposed to xenobiotics (i.e. epithelia of the bronchus, digestive tract, and keratinocytes), in metabolically active tissues (i.e. adrenal cortex), and in macrophages, whereas varying levels were observed in other organs (Izquierdo, 1996a). In agreement with LRP/MVP distribution in normal human tissues, vaults are most abundant in epithelial cells (i.e. rat intestine) and macrophages (i.e. rabbit alveolar macrophages) (Kedersha, 1990). The distribution of LRP/MVP resembles that of other drug resistance-associated proteins such as Pgp and MRP (Flens, 1996; van der Valk, 1990) and suggests a role for vaults in defense against xenobiotics. In support of this view, in normal lung a trend for higher expression of LRP/MVP in tissue obtained from individuals who had smoked more than 10 pack years (1 cigarette pack per day/10 yr) compared with individuals who never smoked has been shown (Dingemans, 1996).

LRP/MVP expression in a panel of tumours

Among 174 tumour specimens comprising 27 tumour types, LRP/MVP was expressed in 63% of the cases (Izquierdo, 1996a). This figure was consistent with the *in vitro* data indicating the widespread distribution of the LRP/MVP-associated mechanism of drug-resistance in human malignancies. It was of interest to note that the distribution of LRP/MVP was not uniform among different tumour types. Rather, LRP/MVP expression closely reflected the susceptibility to chemotherapy of different tumour types. Highly chemosensitive cancers (i.e. germ cell tumours, leukaemias, neuroblastoma) expressed LRP/MVP in a minority of cases, partially chemosensitive cancers (i.e. ovarian carcinoma) in a large proportion of cases, and refractory cancers to chemotherapy (i.e. colon, renal, and pancreatic cancers) were largely LRP/MVP positive (Izquierdo, 1996a). These results further support the potential value of LRP/MVP as a marker of clinical resistance to chemotherapy.

Clinical Value of LRP/MVP to Predict Response to Chemotherapy and Prognoses

Several studies have been initiated in different tumour types to investigate whether the expression of LRP/MVP in clinical specimens is predictive of response to chemotherapy and prognosis. To date, the monoclonal antibody LRP-56 has been used for immunocytochemical, flow cytometry, and immunohistochemical studies. At first optimal staining results with LRP-56 were achieved in frozen tissues as compared to formalin-fixed, paraffin-embedded tissues (Izquierdo, 1996a). Therefore, most of the studies reported so far have used frozen tissues. Recently, the rat monoclonal antibody LMR-5 has been described detecting also the LRP/MVP (Flens, 1997). LRP-56 and LMR-5 recognize different epitopes of LRP/MVP in cell lines and in clinical specimens (Flens, 1997; Schroeijers, 1997). Furthermore, optimized staining protocols for LRP-56 and LMR-5 have been developed which result in the reliable detection of LRP/MVP on paraffin sections (Schroeijers, 1997). The availability of more than one monoclonal antibody and the possibility of using archival tumour material will greatly expedite studies on the putative role of LRP/MVP in clinical drug resistance.

Melanoma

Human malignant melanoma is a highly chemoresistant tumour type, both in *in vitro* models and in the clinic (Garbe, 1993). The mechanism(s) conferring such high drug resistance are largely unknown. Pgp is rarely expressed in melanoma specimens indicating that other mechanisms are responsible for the resistance phenotype of melanoma cells (Schadendorf, 1995). In a series of ten melanoma cell lines, *MDR1*, *MRP*, and *LRP* gene expression at the mRNA level using RT-PCR was detected in 2, 4, and 10 cell lines, respectively (Schadendorf, 1995). Schadendorf *et al.* (1995) studied the immunohistochemical expression of Pgp, MRP and LRP/MVP in 21 primary and 37 metastatic malignant melanoma specimens. Pgp was detected in only one (2%) primary melanoma and MRP in 43% of the tumours with no difference between primary or metastatic lesions or between specimens taken prior to or after chemotherapy. LRP/MVP was expressed in 62% of both primary and metastatic melanoma lesions. However, the number of metastatic specimens that expressed LRP/MVP in > 50% of tumour cells was significantly supe-

rior among those that had been previously exposed to chemotherapy. The frequent expression of MRP and mainly of LRP/MVP, in contrast to Pgp, point to these proteins as potential mediators of the drug resistance phenotype characteristic of malignant melanoma.

Lung cancer

Among the NCI panel, eight out of ten (80%) non-small cell lung cancer (NSCLC) cell lines expressed LRP/MVP, although two displayed LRP/MVP expression in a minority of cells (Izquierdo, 1996b). Dingemans *et al.* (1996) studied the expression of LRP/MVP in 36 non-small cell (NSCLC) and seven small cell (SCLC) lung carcinomas. The expression of LRP/MVP was frequent in NSCLC (86%) and significantly higher as compared to the most chemosensitive subtype SCLC (0%). Among NSCLC, squamous cell and adenocarcinoma had a significant higher expression than the large cell undifferentiated carcinomas. Only 17 patients (ten NSCLC and seven SCLC) received chemotherapy treatment. No correlation between LRP/MVP expression and clinicopathological parameters were found in this small and heterogeneous group of lung cancers. Further studies are needed to elucidate the prognostic significance of LRP/MVP in lung cancer.

Multiple myeloma

The expression of LRP/MVP in multiple myeloma has been reported to be 13 and 48% in two independent studies using immunocytochemistry (H.G.P. Raaijmakers, unpubl.; H. Schwarzenbach, pers. comm.). Schwarzenbach *et al.* found no difference in LRP/MVP expression between myelomas with or without prior chemotherapy. Raaijmakers *et al.* studied 58 previously untreated patients treated with prednisone plus conventional melphalan doses (MP; 38 patients) or melphalan alone at intermediate doses (IDM; 20 patients). The expression of LRP/MVP was associated with a significantly lower response to chemotherapy and shorter survival in the MP group, but not in the IDM group. This finding let the authors to speculate about the possibility that dose intensification of melphalan could overcome LRP/MVP-associated resistance to MP. The prognostic value of LRP/MVP expression in myeloma patients treated with melphalan agrees with the correlation between LRP/MVP expression in 61 cancer cell lines and *in vitro* resistance to melphalan among other MDR-related and MDR-unrelated drugs (Izquierdo, 1996b). However, a

larger study will be necessary to confirm the clinical relevance of LRP/MVP in multiple myeloma.

Neuroblastoma

Neuroblastoma is a relatively chemosensitive tumour type. Izquierdo *et al.* (1996a) showed that, similar to other chemosensitive tumour types, the rate of expression of LRP/MVP was low in neuroblastoma samples from untreated patients, and that the expression was mostly located in mature ganglion cells. Ramani *et al.* (1995) reported that 3 out of 19 (16%) neuroblastoma samples from untreated patients expressed LRP/MVP, as compared with nine out of eleven (82%) samples obtained after chemotherapy treatment. In nine LRP/MVP negative neuroblastomas before chemotherapy, paired samples were available after chemotherapy treatment, and seven of them (78%) converted to LRP/MVP positive (Ramani, 1995). These data suggest an association between exposure to anticancer agents and induction of expression of LRP/MVP. There was a correlation between the degree of differentiation and LRP/MVP expression. LRP/MVP immunoreactivity was also seen in mature ganglion cells. No correlation with other clinico-pathological variables was seen in this small and heterogeneous group of neuroblastoma patients.

Childhood acute leukaemia

The molecular basis of drug resistance in childhood acute lymphoblastic leukaemia (ALL) is largely unknown (Pieters, 1997). Although Pgp is expressed in childhood ALL its role in clinically relevant drug resistance appears to be limited, in contrast to adult acute myeloid leukaemia (List, 1996). *In vitro* resistance to daunorubicin of childhood ALL blast cells was related to prognosis and relapse to prior chemotherapy (Pieters, 1997). In one study including 30 patients with relapsed childhood ALL, the expression of LRP/MVP, but not of Pgp, was significantly associated with an increased *in vitro* resistance of fresh leukaemic cells to daunorubicin (Klumper, 1995). More recently, Veerman *et al.* (1997) reported the expression of Pgp, MRP, and LRP/MVP in initial and relapsed childhood ALL, as well as the accumulation of daunorubicin and sensitivity to daunorubicin and etoposide of leukaemic cells. The accumulation of daunorubicin correlated with *in vitro* sensitivity to this drug.

Expression of Pgp or MRP did not correlate with accumulation nor with sensitivity to daunorubicin.

In contrast, the expression of LRP/MVP was significantly higher in relapsed samples and correlated weakly with *in vitro* resistance to daunorubicin and etoposide. These preliminary studies suggest that expression of LRP/MVP may result in low intracellular concentrations of daunorubicin and point to LRP/MVP as a relevant resistance protein in childhood ALL. Large prospective trials to confirm this possibility are underway. For that, efforts aimed to optimize and standardize the detection methods of Pgp, MRP, and LRP/MVP in blast cells of childhood ALL are welcome (den Boer, 1997).

Adult acute leukaemia (AML)

Among the NCI panel, only one out of six (16%) leukaemia cell lines expressed LRP/MVP (Izquierdo, 1996b). The expression of LRP/MVP in leukaemic cells has been reported in 25 to 37% of AML patients at diagnosis using immunohistochemistry (Borg, unpubl.; Filipits, 1998; Izquierdo, 1996a; List, 1996) or flow cytometry (Michieli, 1997). The studies performed to date show that the expression of LRP/MVP is consistently associated with poor response to induction chemotherapy, as well as with shorter both progression-free and overall survival (Borg, unpubl.; Filipits, 1998; List, 1996).

Most studies, although not all, report that Pgp expression is also associated with poor outcome in AML (List, 1996). Despite the fact that MRP was expressed in *de novo* AML this did not predict for response to chemotherapy or survival (Filipits, 1997). Hart et al (1995) showed that *LRP* mRNA expression, but not *MDR1* mRNA or *MRP* mRNA expression, was significantly increased in patients failing to respond to intensive chemotherapy compared with those achieving complete response. List *et al.* (1996) showed that the prognostic value of LRP/MVP was superior to that of Pgp and, in contrast to Pgp, remained as an independent variable in the multivariate analysis. Co-expression of LRP/MVP and Pgp seems to be present in a relatively small number of *de novo* AMLs (6 to 24%) (Filipits, 1998; List, 1996; Michieli, 1997). In these cases both LRP/MVP and Pgp seem to contribute to the resistance phenotype. The response to chemotherapy was best in AML patients lacking expression of both genes, intermediate in those with expression of either of these two proteins, and worst in patients expressing both genes (Filipits, 1998; List, 1996). In addition, disease-free and overall survival were significantly longer for patients with LRP/MVP-

and Pgp-negative AMLs (Filipits, 1998). These data show that expression of LRP/MVP is a prognostic factor in AML and point to LRP/MVP as a clinically relevant drug resistance gene in AML. However, the functional implications of the expression of LRP/MVP and the prospect that vaults play a role in drug transport need to be elucidated. In support of this possibility, leukaemic blast cells which overexpress LRP/MVP, but not Pgp, showed reduced intracellular daunorubicin accumulation (Michieli, 1997). Leukaemic cells overexpressing only Pgp had a defect in both daunorubicin accumulation and rhodamine 123 retention. Interestingly, leukaemic cells with LRP/MVP and Pgp overexpression had also impaired daunorubicin accumulation, but displayed variable and unforeseeable capacity of effluxing rhodamine 123 suggesting complex interactions between LRP/MVP and Pgp (Michieli, 1997). Strategies aimed to overcome drug resistance in AML patients should contemplate the use of resistance modifiers not only of Pgp function, but also of vaults function.

Ovarian cancer

The expression of LRP/MVP in the tumours of 57 women with FIGO stage III/IV ovarian cancer indicated that LRP/MVP positive tumours had a significantly inferior response to platinum and alkylating agent based chemotherapy (Izquierdo, 1995). Furthermore, the expression of LRP/MVP was significantly associated with a shorter interval until tumour progression and shorter overall survival. These clinical data are supported by *in vitro* data showing the association between LRP/MVP expression and intrinsic or acquired resistance to cisplatin among the 61 cell lines of the NCI panel (Izquierdo, 1996b) or in A2780/CP70 human ovarian cancer cells selected with cisplatin (Parker, 1997), respectively.

The clinico-pathologic studies reviewed above agree, in general, in establishing LRP/MVP as a potential useful marker of drug resistance in the clinic. The fact that LRP/MVP, rather than Pgp or MRP, shows strong prognostic value in different cancers treated with dissimilar chemotherapeutic regimens is certainly remarkable. These results agree with the capacity of LRP/MVP to predict a broad resistance phenotype in the NCI panel which is composed of eight different cancer types (Izquierdo, 1996b). This might indicate a direct role for vaults in drug resistance to both classical and non-classical MDR drugs, or alternatively,

Table 1. Relationship between LRP expression in human tumors and response/follow up data

Tumor type ^a	Technique ^b	LRP positive	LRP expression and response ^{c,d}	Reference
Hematological malignancies				
AML	IHC	28/69	shorter CR and PR, with shorter PFS (p<0.05)	List <i>et al.</i> , 1996
AML	RT-PCR	8/30	decreased response to chemotherapy (p<0.05)	Hart <i>et al.</i> , 1995
AML	IHC	de novo 18/52 relapsed 14/16	reduced CR (P=0.00002) and OS (P=0.01)	Borg <i>et al.</i> , 1996
AML	IHC	31/86	shorter CR (p=0.01), OS (p=0.01) and DFS (p=0.03)	Filipits <i>et al.</i> , 1998
Leukemias	FCM	23/65 (16/65 Pgp and LRP)	decreased intracellular drug-accumulation (p<0.001)	Michieli <i>et al.</i> , 1997
Childhood ALL	FCM	no detailed data	increased <i>in vitro</i> daunorubicin resistance (MTT-assay) (p<0.016)	den Boer <i>et al.</i> , 1996
Multiple Myeloma	IHC	28/58	shorter median survival, poor response to melphalan (p<0.002)	Raaymakers <i>et al.</i> , 1997
Solid tumors				
Melanoma	IHC	13/21 primary tumors, 23/37 metastases	more frequent after chemotherapy (p<0.05)	Schadendorf <i>et al.</i> , 1995
NSCLC	IHC	36/36: >10% cells	not a prognostic factor for survival	Dingemans <i>et al.</i> , 1996
SCLC	IHC	7/7: < 10% cells	not associated with clinical outcome (NS)	
Ovarian carcinoma	IHC	44/57	reduced PFS and OS (p<0.005)	Izquierdo <i>et al.</i> , 1995

^a Malignancies: AML (acute myeloid leukemia), ALL (acute lymphocytic leukemia), (N)SCLC (non-) small cell lung cancer.

^b Technique: RT/PCR (reverse transcriptase/polymerase chain reaction). IHC (immunohistochemical staining), FCM (flow-cytometry).

^c Response: CR/PR (complete response/partial response), PFS (progression-free survival time in months), OS (overall survival), DFS (disease free survival).

^d p-value as reported by the authors, NS (not significant).

that LRP/MVP is frequently co-expressed with other resistance mechanisms. However, besides the value of LRP/MVP as a marker of drug resistance, the potential functional implication of vaults in drug resistance remains to be elucidated.

Possible Mechanism of Vault-Related Drug Resistance

A plausible hypothesis regarding the functional role of vaults in drug resistance results from the observation of drug re-distribution in MDR cancer cell lines and leukaemic blast cells and from the data on structure and cellular localization of vaults (Izquierdo, 1996c).

Drug re-distribution in MDR cancer cells

MDR cells often display an altered intracellular distribution of drugs as compared to parental cells (Coley, 1993; Schuurhuis, 1989). MDR cells distribute daunorubicin into the perinuclear region and, subsequently, redistribute the drug away from the nu-

cleus into a punctate cytoplasmic pattern, whereas parental cells localise daunorubicin in a diffuse nuclear and cytoplasmic pattern (Gervasoni, 1991; Hindenburg, 1989). This phenomenon has been observed in LRP/MVP overexpressing MDR cancer cells (Schuurhuis, 1989). In certain cell lines, drug re-distribution is accompanied by the formation of vesicular structures (Dietel, 1990).

Drug re-distribution in leukaemic blast cells

Michieli *et al.* (1997) reported that leukaemic blast cells which only expressed LRP/MVP, but not Pgp or MRP, showed an impaired intracellular accumulation of daunorubicin and suggested that vaults could be implicated in this phenomenon.

Vault location and structure

Vaults are good candidates to be the perinuclear and cytoplasmic structures mediating daunorubicin re-distribution within MDR cells and leukaemic blast cells. Vaults are located at the NPCs possibly composing, indeed, the transporter units (Chugani, 1993;

Rome, 1991). Cytoplasmic vaults have been shown to be associated with vesicles from rat liver and synaptic vesicles in nerve terminals, although it is not yet clear whether there is a real interaction between both structures (Herrmann, 1996; Kedersha, 1986).

Hypothesis

It is tempting to hypothesize that vaults can mediate drug resistance by regulating both the nucleocytoplasmic transport of drugs and their cytoplasmic redistribution within vesicles keeping cytotoxic agents away from their cellular targets (Izquierdo, 1996c). Studies to corroborate this hypothesis are underway. Transfection of the *LRP* gene alone has failed to confer MDR, an expected finding considering that the complete vault particle will be required for functional activity (Scheffer, 1995). Ribozyme and antisense approaches aimed at disruption of vaults function are at present under investigation to clarify the functional role of vaults in drug resistance.

Concluding Remarks

The understanding of mechanisms mediating MDR is fundamental to develop strategies aimed to prevent or circumvent cytostatic drug resistance, thus improving results of chemotherapy. The data reviewed indicate that the presence of vaults, as determined by LRP/MVP expression, appears to be emerging as a valuable marker of drug resistance in both acquired and intrinsically resistant cancer cell lines, as well as in cancer patients. Furthermore, the data suggest that vaults may constitute a novel mechanisms of drug resistance. Certainly, full proof for whether and how this mechanism actually represents drug transport still has to be generated. Nevertheless, both the molecular, genetic and clinical data obtained so far warrant further studies into the role of LRP/MVP, vault-related drug resistance. Subsequent studies may then lead to the identification of molecules able to prevent or antagonize this type of resistance.

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