Relationship of LRP-human major vault protein to \textit{in vitro} and clinical resistance to anticancer drugs

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\textbf{Abstract}

Multidrug resistance (MDR) has been related to two members of the ABC-superfamily of transporters, P-glycoprotein (Pgp) and Multidrug Resistance-associated Protein (MRP). We have described a 110 kD protein termed the Lung Resistance-related Protein (LRP) that is overexpressed in several non-Pgp MDR cell lines of different histogenetic origin. Reversal of MDR parallels a decrease in LRP expression. In a panel of 61 cancer cell lines which have not been subjected to laboratory drug selection, LRP was a superior predictor for \textit{in vitro} resistance to MDR-related drugs when compared to Pgp and MRP, and LRP’s predictive value extended to MDR unrelated drugs, such as platinum compounds. LRP is widely distributed in clinical cancer specimens, but the frequency of LRP expression inversely correlates with the known chemosensitivity of different tumour types. Furthermore, LRP expression at diagnosis has been shown to be a strong and independent prognostic factor for response to chemotherapy and outcome in acute myeloid leukemia and ovarian carcinoma (platinum-based treatment) patients. Recently, LRP has been identified as the human major protein. Vaults are novel cellular organelles broadly distributed and highly conserved among diverse eukaryotic cells, suggesting that they play a role in fundamental cell processes. Vaults localise to nuclear pore complexes and may be the central plug of the nuclear pore complexes. Vaults structure and localisation support a transport function for this particle which could involve a variety of substrates. Vaults may therefore play a role in drug resistance by regulating the nucleocytoplasmic transport of drugs.

\textbf{Abbreviations:} LRP – Lung Resistance-related Protein; MVP – Major Vault Protein; MDR – Multidrug resistance; MRP – Multidrug resistance-associated Protein; NPC – Nuclear Pore Complex, Pgp – P-glycoprotein.

\textbf{Introduction}

Broad resistance to chemotherapeutic drugs is a major cause of failure of cancer treatment. \textit{In vitro} this phenomenon can occur as a result of exposure of cancer cells to a single cytotoxic drug and is termed multidrug resistance (MDR) (Childs and Ling, 1994). MDR has been associated with the overexpression of P-glycoprotein (Pgp) or Multidrug Resistance-associated Protein (MRP) (Childs and Ling, 1994). However, there is increasing evidence from \textit{in vitro} and clinical studies that additional mechanisms of MDR may be operative. A novel protein associated with MDR, originally termed the Lung Resistance-related Protein (LRP), has been described (Scheper, 1993). This article reviews the data supporting the association of LRP with drug resistance \textit{in vitro}, its distribution in normal human tissues, as well as its potential value as a marker.
of clinical drug resistance. In addition, the molecular characterization of LRP and the possibility that LRP may be involved in an entirely new mechanism of drug resistance is discussed.

Identification of the Lung Resistance-related Protein (LRP) in SW-1573 non-small-cell lung cancer MDR sublines

A series of MDR sublines was developed from the SW-1573 non-small cell lung cancer cell line by exposure to increasing concentrations of doxorubicin. The 2R120 subline showed moderate levels of resistance to doxorubicin, vincristine, and etoposide (4- to 45-fold), in the absence of Pgp expression (Kuiper, 1990). The 2R120 cell line was chosen to investigate non-Pgp-mediated MDR. BALB/c mice were immunized with 2R120 cells and the monoclonal antibody LRP-56 was selected for strong immunoreactivity with 2R120 cells compared to parental SW-1573 cells (Scheper, 1993). LRP-56 specifically reacted with a 110 kDa protein, LRP, which was overexpressed in 2R120 cells. The SW-1573/2R120 revertant cell line (2R120 cell line cultured without drug for over nine months) showed a decrease in the level of resistance to approximately equal levels to the parental cells. Of interest, this cell line also showed a decrease in the level of LRP expression, further supporting a close association between LRP and drug resistance in 2R120 cells. Furthermore, the 2R160 cell line, a SW-1573 subline displaying high levels of resistance and increased MDR1/Pgp expression had very low LRP expression, similar to the parental SW-1573 cells (Scheper, 1993).

LRP-56 displays a characteristic cytoplasmic punctate staining pattern in 2R120 cells (Scheper, 1993). This staining pattern is conserved in other MDR cell lines, in normal human tissues, and in human malignancies.

LRP overexpression in MDR cancer cell lines

The overexpression of LRP in the 2R120 cell line was found not to be a peculiarity of these cells, but a more general feature of non-Pgp-mediated MDR. LRP has also been found to be overexpressed in a number of other Pgp-negative MDR cell lines of different histogenetic origin (Scheper, 1993). These include cell lines derived from small cell lung cancer (GLC4/ADR), fibrosarcoma (HT1080/DR4), breast cancer (MCF7/Mitox), and myeloma (8226/MR40). The up-regulation of LRP occurs early during the process of drug selection in various series of MDR sublines, such as those derived from the SW-1573, and GLC4 cells (Scheper, 1993; Versantvoort, 1995). This observation suggests that the LRP-associated mechanism is already involved in low or moderate levels of drug resistance, which are likely to be more clinically relevant.

Most LRP overexpressing MDR cell lines also display increased levels of MRP (Scheper, 1993; Flens, 1994). Although these two MDR-related proteins are frequently co-upregulated in MDR cell lines, there is evidence that both genes can be regulated independently (see below). Since transfection of the MRP gene itself has been associated with low or moderate levels of drug resistance (Zaman, 1994), the concomitant operation of several drug resistance mechanisms may be necessary to achieve the high levels of resistance observed in most LRP and MRP positive MDR cells. In contrast, most Pgp-positive MDR cell lines do not overexpress LRP, like the non-small cell lung cancer SW-1573/2R160, the ovarian carcinoma A2780AD, and the myeloma 8226/Dox 6 and 8226/Dox40 cell lines (Scheper, 1993). However, LRP and Pgp expression are not always mutually exclusive. The MCF7/D40 breast cancer cell line and certain 8226 myeloma sublines showed increased levels of both Pgp and LRP (Scheper, 1993; Shao, 1995). Despite the similar Pgp content observed in independently isolated 8226 MDR sublines, the resistance levels were substantially higher in a Pgp/LRP positive 8226 subline than in the Pgp positive/LRP negative 8226/Dox 6 subline, suggesting that LRP has functional relevance in certain Pgp overexpressing MDR cells (Shao, 1995). Remarkably, LRP overexpression has been reported in Pgp/MPR negative MDR cell lines, such as the mitoxantrone selected MCF7/MR cell line (Futschier, 1994). In these cells, the LRP-associated mechanisms of MDR may play a prominent role.

Relation of LRP to drug resistance in human cancer cell lines not selected in the laboratory for drug resistance

Studies on the mechanisms of resistance have concentrated on laboratory selected MDR cancer cells generated through a stringent drug-treatment selection procedure, and in general, show (very) high levels of resistance. Therefore, it may be difficult to extrapolate the