Transporter molecules in multidrug resistance

Rik J. Scheper, George L. Scheffer, Marcel J. Flens, Paul van der Valk, Henk J. Broxterman and Miguel A. Izquierdo
Departments of Pathology (RJS, GLS, MJF, PVDV, MAI) and Medical Oncology (HJB), Free University Hospital, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

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Introduction

Multidrug resistance in clinical cancer is often associated with reduced intracellular concentrations of cytostatic drugs. This phenomenon may be caused by several different mechanisms, including overexpression of members of the ABC-superfamily of transmembrane transporter molecules, such as P-glycoprotein (Pgp) and multidrug-resistance related protein (MRP), but probably also by overexpression of the transporter associated with antigen-presentation (TAP), and overexpression of vault particles (Vault-Related MDR). Since 1987 we have studied these mechanisms by producing several monoclonal antibodies (mAbs), subsequently followed by in vitro studies with tumor cell lines selected for drug-resistance, evaluation of normal human tissue distribution patterns, and studies in clinical cancer.

P-glycoprotein (Pgp)

We succeeded in generating the Pgp-specific mAb JSB-1 by immunizing mice with the tumor cell line CHrC5, at that time already known to overexpress Pgp (Scheper et al., 1988). Since the epitope was found to be highly specific for the mdr1 gene product, and not to cross-react with the closely related mdr3 gene product, JSB-1 has since been used in numerous experimental and clinical studies on Pgp-mediated MDR. Moreover, in later studies optimal conditions could be defined allowing its use on formaldehyde fixed, paraffin-embedded tissues (Toth et al., 1994; Itsubo et al., 1994). In in vitro studies with various couples of parental (drug-sensitive) and selected (drug-resistant) tumor cell lines, pharmacokinetic aspects of Pgp-function could be elucidated. Subsequently the normal tissue distribution of Pgp was elucidated (van der Valk et al., 1990).

In clinical studies on haematological malignancies, JSB-1 and other anti-Pgp mAbs have been used to identify patients poorly responding to chemotherapy, whereas Pgp-overexpression was also found to predict for potential benefit of the use of the Pgp-blocker verapamil (Simpson et al., 1985; Dalton et al., 1989, Sonneveld et al., 1994). Currently panels including at least two different Pgp-specific mAbs are widely used for clinical diagnostic purposes, and studies on the potential role of Pgp in clinical drug resistance in solid tumor types. Generally, such a role seems limited to distinct tumor types, and since 1992 much attention has shifted to the newly discovered MRP.

Multidrug-resistance related protein (MRP)

In 1992 Cole et al. (1992) described a gene encoding another ABC transporter molecule which they named multidrug-resistance associated protein (MRP). In collaboration with Dr. G. Zaman (Neth. Cancer Inst.) we prepared two bacterial fusion proteins containing different fragments of MRP. The first fusion protein contained the amino-proximal half of the the protein. The second contained the carboxyl-terminal end and part op the predicted carboxyl-proximal nucleotide binding domain of MRP. After immunization with the fusion porteins we succeeded in the development of MRP-
specific polyclonal (Zaman et al., 1994) and monoclonal antibodies: the mouse mAb MRP-m6 and rat mAb MRP-r1 (Flens et al., 1994). The specificity of the antisera was confirmed on protein blots and cyto-centrifuge preparations of various tumor cells, including cell lines transfected with either the MRP or the MDR1 genes.

The MRP-specific antibodies allowed us to further characterize transfec tant cell lines and to reveal that MRP, like Pgp, is primarily expressed at the outer cellular membrane (Zaman et al., 1994). Studies by Müller et al. (1994) indicated that MRP functions as an ATP-dependent plasma-membrane efflux pump for glutathione-S-conjugates. Studies on the development of clinical applicable drugs to reverse MRP-mediated MDR have been initiated. The glutathione-synthesis blocker buthionine sulfoximine could provide a new lead for resistance-modifying agents in this type of resistance (Versantvoort et al., 1995).

Recently, using both the newly developed mAbs and cDNA-probes for RNAse protection assays, we have completed a survey of MRP-expression in normal human tissues. The tissue distribution of MRP showed staining in different epithelial cells including cells of the bronchus of the lung, the digestive tract, the urinary bladder, and the adrenal cortex. Also alveolar macrophages and stromal cells in various organs showed immunoreactivity (Flens et al., 1996). Based on the presence of MRP in many epithelia, MRP, like Pgp, may play a role in the protection of the organism against xenobiotics. Furthermore MRP may have a role in the secretion of hormones (Flens et al., submitted). At this stage little data is available confirming relevance of MRP overexpression in clinical drug resistance. Recently overexpression of MRP was reported in AML (Schneider et al., 1995; Schuurhuis et al., 1995).

**LRP/Vault-related multidrug-resistance (LRP/VR-MDR)**

Using a Pgp-negative tumor cell line for immunization, we have raised the monoclonal antibody (MAb) LRP-56, recognizing a ≈ 110kD protein which we named Lung Resistance Protein (LRP; Scheper et al., 1993; see also chapter by Izquierdo et al., in this book). After cloning the LRP gene, analysis of the deduced amino acid sequence indicated that the LRP-gene product is a 100kD protein showing 91% identity with the major vault protein from the rat (Scheffer et al., 1995). Rat vaults are ribonucleoprotein particles that contain a major vault protein of 104kD which accounts for > 70% of the particle, three minor proteins of 210, 192, 54 kD, and a small RNA molecule. These components are assembled in a complex barrel-shaped structure and compose the largest ribonucleoprotein body reported to date. Vaults are associated with vesicular fractions in the cytoplasm and with the nuclear pore complexes (NPCs). Structural studies indicate that, within the nuclear pore complexes, vaults form the NPCs transporter units, therefore, implicating vaults in nucleocytoplasmic transport of various substrates (Rome et al., 1991). Interestingly, drug-accumulation studies show decreased nucleus/cytoplasm ratios in LRP overexpressing cells (Schuurhuis et al., 1995). Vesicle-associated vaults might play a role in entrapping of toxic agents into exocytotic vesicles. In acute adult myeloid leukemias and in ovarian carcinoma we

**Transporter associated with antigen-presentation (TAP)**

We also investigated the potential role in MDR of another ABC-transporter, the transporter associated with antigen-presentation (TAP). TAP-heterodimers translocate peptides from the cytosol to the ER-lumen where association with MHC-Class I molecules occurs. Using an anti-TAP-antiserum on immunochemistry and immunoblots, we found TAP overexpression in 3/3 MRP+ MDR cell lines, and in 2/5 Pgp+ MDR cell lines. TAP-expression was reduced in tumor cells which had been cultured without drug and had lost drug resistance. In all cell lines MHC Class I expression closely paralleled TAP-expression (Izquierdo et al., submitted).

In drug-sensitivity assays T1 (TAP+), as compared to T2 (TAP-), tumor cell lines showed approximately 2-fold resistance to VP-16, vincristine and doxorubicin. Resistance was restored to T1 levels in T2 cells cotransfected with both TAP genes. Furthermore, in a competition assay, VP-16 inhibited TAP translocation to the ER of a radiolabelled model peptide. The results demonstrate that TAP modestly contributes to MDR, specially in Pgp/MRP+ MDR tumor cell lines, where it may cooperate with MRP (Izquierdo et al., submitted). Of great interest will be to study the TAP-mediated effect of cytostatic drug treatment for augmenting MHC Class I expression on tumor cells as an approach to facilitate cytotoxic T cell-mediated immunotherapies.