

Vaults: a ribonucleoprotein particle involved in drug resistance?

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Vaults are ribonucleoprotein particles found in the cytoplasm of eucaryotic cells. The 13MDa particles are composed of multiple copies of three proteins: an M_r 100 000 major vault protein (MVP) and two minor vault proteins of M_r 193 000 (vault poly-(ADP-ribose) polymerase) and M_r 240 000 (telomerase-associated protein 1), as well as small untranslated RNA molecules of approximately 100 bases. Although the existence of vaults was first reported in the mid-1980s no function has yet been attributed to this organelle. The notion that vaults might play a role in drug resistance was suggested by the molecular identification of the lung resistance-related (LRP) protein as the human MVP. MVP/LRP was found to be overexpressed in many chemoresistant cancer cell lines and primary tumor samples of different histogenetic origin. Several, but not all, clinico-pathological studies showed that MVP expression at diagnosis was an independent adverse prognostic factor for response to chemotherapy. The hollow barrel-shaped structure of the vault complex and its subcellular localization indicate a function in intracellular transport. It was therefore postulated that vaults contributed to drug resistance by transporting drugs away from their intracellular targets and/or the sequestration of drugs. Here, we review the current knowledge on the vault complex and critically discuss the evidence that links vaults to drug resistance.

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Vaults: a conserved organelle

The vault complex, a large-sized ribonucleoprotein, was first described in the mid-1980s (Kedersha and Rome, 1986). The barrel-shaped structures were initially detected in preparations of clathrin-coated vesicles from rat liver, and because they displayed a morphology that resembled the vaulted ceilings in cathedrals, the structures were named vaults. It is now known that structures of similar dimension, morphology and composition are present in the cells of diverse eucaryotic

organisms like protozoa, molluscs, the slime mold *Dictyostelium discoideum*, echinoderms, fish, amphibians, avians and mammals (Kedersha *et al.*, 1990; Rome *et al.*, 1991). However, vaults could not be detected in *Saccharomyces cerevisiae* (Kickhoefer *et al.*, 1996) and are probably not present in *Caenorhabditis elegans*, *Drosophila melanogaster* and the plant *Arabidopsis* sp, that is, no clear vault protein orthologs could be detected in the genomes of these organisms. Nevertheless, the high degree of evolutionary conservation of the complex implies an important cellular function.

Components of the vault complex

The mammalian vault complex consists of multiple copies of three proteins of M_r 100 000, 193 000 and 240 000, respectively, and small untranslated RNA molecules of 88–141 bases. The M_r 100 000 major vault protein (MVP) was found to be identical to the previously described lung resistance-related protein (LRP) (Scheffer *et al.*, 1995), and makes up over 70% of the total mass of the complex. Emphasizing the importance of MVP for the vault structure is the fact that the expression of MVP in vault-lacking insect cells is sufficient for the assembly of vault-like particles (Stephen *et al.*, 2001). At least two distinct domains can be distinguished in human MVP: a coiled-coil domain at its C-terminal end that is responsible for the interaction between two MVP molecules, and thereby crucial for vault formation (van Zon *et al.*, 2002). In addition two, and possibly three, calcium-binding EF hands have been identified in a degenerated 50 amino-acid repeat structure in the N-terminal half of the molecule (van Zon *et al.*, 2002). The interaction of MVP or vaults with other proteins might be mediated by calcium (Yu *et al.*, 2002). The primary sequence of MVPs from several different species is known and appears to be highly conserved, with an overall identity of ~90% between mammalian MVPs, which still have a considerable identity (~60%) with MVPs from most lower eucaryotes (Table 1). Similarly, the intron–exon structure of the human and mouse MVP genes is conserved as is their 5' region, comprising the first untranslated exon and 400 base pairs of upstream sequence, which was shown to exhibit promoter activity (Lange *et al.*, 2000; Mossink *et al.*, 2002a).

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Table 1 Percentages of overall amino-acid identity between major vault proteins from several organisms

Name/# aa res.	Human	Mouse	Rat	T. marmorata	I. punctatus	D. discoideum A	D. discoideum B	M. edulis
HsMVP/893	—	—	—	—	—	—	—	—
MmMVP/861	91	—	—	—	—	—	—	—
RnMVP/895	87	95	—	—	—	—	—	—
TmMVP/852	68	68	68	—	—	—	—	—
IpMVP/549	67	65	65	65	—	—	—	—
DdMVPA/843	57	56	56	55	55	—	—	—
DdMVPB/846	52	52	52	51	50	60	—	—
MeMVP/279 ^a	58	58	58	60	64	56	49	—
LmMVP/1074 ^b	37	37	35	37	34	40	40	31

Table adapted from Mossink *et al.* (2002a). Abbreviations: # aa res., number of amino-acid residues; HsMVP, *Homo sapiens* MVP/Genbank acc. no. X79882; MmMVP, *Mus musculus* MVP/AF210456; RnMVP, *Rattus norvegicus* MVP/U09870; TmMVP, *Torpedo marmorata* MVP/X87771; IpMVP, *Ictalurus punctatus* MVP/AAG00866; DdMVPA, *Dictyostelium discoideum* MVP A/L08646; DdMVPB, *D. discoideum* MVP B/Z37109; MeMVP, *Mytilus edulis* MVP/AF172605; LmMVP, *Leishmania mexicana* MVP/AL445944. ^aNote that the *M. edulis* sequence deposited in Genbank is incomplete. ^bTranslation start is not mapped and *L. mexicana* MVP may be shorter, starting at M₁₁₅

The M_r 193 000 vault subunit was named vault poly-(ADP-ribose) polymerase (VPAAP), because it contains a functional poly-(ADP-ribose) polymerase (PARP) domain (Kickhoefer *et al.*, 1999a). VPAAP was capable of ADP-ribosylating itself and the MVP, but this activity was not yet shown to be of functional importance within the vault complex. So far, seven additional human proteins with PARP activity have been described (Shall, 2002). The prototype of this family of PARP proteins is PARP-1. This nuclear enzyme was shown to bind tightly to nicked DNA and appears to be involved in the base excision repair pathway (for a review see Oliver *et al.*, 1999). Also other members of this group of proteins seem to function in the maintenance of genomic stability either by involvement in DNA repair pathways like PARP-2 (Schreiber *et al.*, 2002) or by acting as telomere-length regulators like tankyrase-1 and -2 (Smith *et al.*, 1998; Sbodio *et al.*, 2002). Three additional family members PARP-3, PARP-6 and PARP-7 have not yet been well characterized (Johansson, 1999; Ma *et al.*, 2001; Shall, 2002). Despite an overall similarity of 29–60% between their PARP domains, the PARP proteins do not resemble each other outside this domain (Smith, 2001). The unique features of each of these proteins may point to different cellular functions. Alternatively, the PARP activity that they display may be an important regulatory mechanism that operates at different levels in the same cellular pathway. Interesting in this context is that VPAAP is also present inside the nucleus, where it is not associated with other vault components (Kickhoefer *et al.*, 1999a). Moreover, VPAAP contains a BCRT domain, a widespread motif in proteins involved in DNA damage repair, which is also used by PARP-1 for its interaction with the base excision repair complex protein XRCC1 (Masson *et al.*, 1998). Similar to other PARP proteins VPAAP, and possibly vaults, may therefore play a role in DNA repair. Clearly, it is important in this respect to establish whether vaults and the nonvault-associated VPAAP are functionally related. Analysis of MVP knockout tissues indicated that the absence of MVP resulted in dramatically lowered cellular VPAAP levels (Mossink *et al.*, 2002b). Note that

vaults contributing to genomic stability in this way could possibly confer a certain level of drug resistance.

The M_r 240 000 vault protein was identified as telomerase-associated protein (TEP1), a protein previously found to be associated with the telomerase complex (Harrington *et al.*, 1997; Kickhoefer *et al.*, 1999b). Within the telomerase complex, the function of TEP1 is still unknown. It was shown to interact specifically with the telomerase RNA (TR) (Harrington *et al.*, 1997) and also with the vault RNAs (vRNAs) (Kickhoefer *et al.*, 1999b). Since only two components of the telomerase complex seem essential for its function *in vitro*, namely TERT and the TR, TEP1 was thought to be a structural component (Weinrich *et al.*, 1997; Beattie *et al.*, 1998). Indeed analysis of a TEP1-deficient mouse model showed that this protein is not essential for telomerase activity. Moreover, telomere length was also unaffected after disruption of TEP1 (Liu *et al.*, 2000). Seemingly normal vault particles could readily be isolated from the tissues of TEP1 knockout mice; however, a closer examination by cryoelectron microscopy revealed a decreased electron density at the extreme ends of the caps of the vault structure (Kickhoefer *et al.*, 2001). Whereas absence of TEP1 did not influence the levels of TR associated with the telomerase complex, the association of vRNA with the vault complex was completely disrupted. In addition, the level of vRNA and its stability were found to be markedly decreased in TEP1 knockout tissues. The biological significance of TEP1 as a shared subunit for both the telomerase complex and vault complex is not clear. Should TEP1 be interpreted as a link between the two ribonucleoprotein complexes, or do both complexes simply use features of the TEP1 protein, for example, its RNA-binding capability for their own purpose?

The vRNA constitutes less than 5% of the mass of the complex and is believed to be a functional rather than a structural component, as degradation of the vRNA did not affect the vault morphology (Kedersha and Rome, 1986; Kong *et al.*, 2000; Liu *et al.*, 2000). Interestingly, in human cells three related vRNAs are expressed, namely hvgl, 2 and 3, of 98, 88 and 88 bases in size, respectively. The hvgl genes are arranged in a triple

repeat structure on chromosome 5 and probably arose through gene duplication. Other species like rats and mice only express one vRNA of 141 bases, and two vRNAs of 89 and 94 bases are found in bullfrog (Kickhoefer *et al.*, 1993, 1998; van Zon *et al.*, 2001). In all vRNAs, the typical internal polymerase III promoter elements are highly conserved. The reason for the existence of multiple vRNAs in some species is unknown. The functional range of the relatively long rodent vRNA might in humans be covered by three shorter vRNA versions. It was shown that all three human vRNAs are bound to the vault complex, but not in a ratio that reflected their expression levels. Apparently, the vRNAs have different affinities for TEPI. The bulk of vRNA associated with the vaults is hvgl and only small amounts of hv2 and 3 could be detected. Interestingly, it was found that in at least three drug-resistant, vault-expressing cell lines, relatively more hv3 was associated with the vaults (van Zon *et al.*, 2001), suggesting that the ratio in which vRNA species are associated to vaults may be of functional significance. In recent years, many novel small nonprotein coding RNAs have been identified. Their structural versatility and ability to interact via hydrogen bonding with specific sequences in other nucleic acids makes them suitable for a diverse range of biological functions from structural to regulatory to catalytic. Further studies investigating the binding of vRNA by TEPI as well as the generation of a vRNA knockout may help to reveal the function of vRNA and its significance for the vault complex.

Structure of the vault complex

The vault complex appears to be 42×75 nm in size and has an estimated molecular mass of 13 MDa (Kedersha *et al.*, 1991), making it the largest ribonucleoprotein complex known to date. Early electron micrographs showed that vaults have two centers of mass, suggesting that the vault complex consists of two symmetrical halves. Indeed, it was shown that the complex can fall apart in two halves that can unfold into flower-like structures (Kedersha *et al.*, 1991). Each flower consists of eight distinct petals that are joined to a central ring. Recently, cryoelectron microscopy combined with three-dimensional image reconstruction techniques revealed the vault structure at a 22 Å resolution (Kong *et al.*, 1999, 2000). Vaults appear to be hollow barrel-like structures with an 8–2–2 symmetry. Each vault particle has an invaginated waist and two protruding caps (Figure 1). The minor vault proteins TEPI – and probably VPARP – as well as the vRNAs are localized in the protruding caps of the vault complex (Kickhoefer *et al.*, 1999b; Kong *et al.*, 2000). A stoichiometric model has been proposed in which each vault consists of 96 copies of MVP, two molecules of TEPI, eight molecules of VPARP and three or more copies of vRNA (Kong *et al.*, 2000).

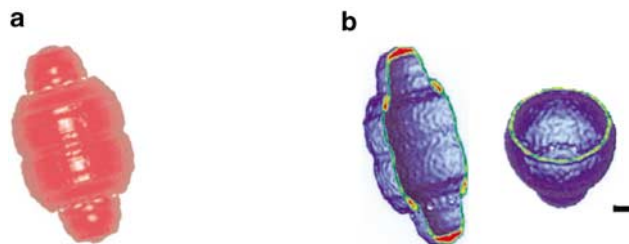


Figure 1 Reconstruction of the vault complex at a 22 Å resolution. Using single-particle reconstruction techniques, approximately 3500 particle images obtained by cryoelectron microscopy were combined to generate the three-dimensional image of the vault complex (a), including two cropped views (b). Clearly visible is the symmetrical hollow barrel-shaped structure and two protruding caps. Note that the vault complexes used were RNase treated, resulting in a slightly reduced density at the end of the caps. The scale bar corresponds to 130 Å. Figure reproduced from Kong *et al.* (2000) with permission from the Cambridge University Press

Intracellular localization of vaults

The number of vaults per cell has been estimated to be as many as 10 000–100 000 copies (Kickhoefer *et al.*, 1998). The majority of these reside in the cytoplasm where they may interact with cytoskeletal elements like the ends of actin stress fibers in rat fibroblasts (Kedersha and Rome, 1990) and in the tips of differentiated PC12 cells (Herrmann *et al.*, 1999) or microtubules (Hamill and Suprenant, 1997). Several groups reported the association of vaults with the nucleus, in particular the nucleoli, the nuclear membrane and/or the nuclear pore complex (Chugani *et al.*, 1993; Hamill and Suprenant, 1997; Abbondanza *et al.*, 1998). In general, in mammalian cells, not more than 5% of the total vault fraction is found associated with the nucleus.

Function of vaults

Despite the characterization of individual vault components and the development of a detailed structural model in recent years, the cellular function of vaults has still not been elucidated. A role in intracellular transport, in particular nucleocytoplasmic transport, has been proposed by several investigators based on the subcellular localization and typical structure of the complex. The partial colocalization of vaults with cytoskeletal elements (Kedersha and Rome, 1990; Hamill and Suprenant, 1997; Herrmann *et al.*, 1999) and the location of vaults near secretory organelles in nerve growth factor treated PC12 neuron-like cells (Herrmann *et al.*, 1999) has led to the hypothesis that vaults can be transported along cytoskeletal elements, in particular microtubuli. This was supported by a report showing that vaults are actively transported within axons between the soma and the nerve terminal (Li *et al.*, 1999). Cytoskeletal-mediated transport would certainly enable vaults to shuttle cargo directionally to specific locations in the cell. However, to prove the existence of

such a transport convincingly, additional studies are necessary addressing vault dynamics, for example, by using a tagged vault complex and investigating the effects of microtubule (de)stabilizers, the energy dependence of transport and the involvement of molecular motors. The idea of vaults specifically taking part in a nucleocytoplasmic transport route was based on observations in rat fibroblasts in which vaults were detected in close proximity to the nuclear pore complex (Chugani *et al.*, 1993). The initial suggestion that vaults were in fact the elusive central plug, which is often observed in the nuclear pore, is probably not correct. The central plug is now generally regarded as consisting of material in transit through the nuclear pore rather than as a separate physical entity (see e.g. Stoffler *et al.*, 1999). The observations made by Chugani and others may in fact represent vaults docking at the nuclear pore in order to take up or give off cargo. The question arises as to the nature of their cargo. Studies in developing sea urchin embryo's where MVP was copurified with ribosomes led to the suggestion that vaults are involved in the transport of ribosomes (Hamill and Suprenant, 1997). However, so far no further studies corroborated this hypothesis. Vaults were also indicated in the nuclear targeting of steroid hormone receptors, most notably the estrogen receptor, and hence may play a role in the signal transduction of steroid hormones (Abbondanza *et al.*, 1998). Coimmunoprecipitation experiments and domain mapping showed that the estrogen receptor binds via its nuclear localization signal to the outside of the vault complex. The hollow structure of the complex fits well with the idea of vaults being involved in cellular transport (Kong *et al.*, 1999). The internal cavity is about $5 \times 10^7 \text{ \AA}^3$ in size, making it large enough to contain particles such as ribosomes. Indeed, cryoelectron microscopical images often show electron dense material within the isolated vault particles (Kong *et al.*, 1999). The characterization of the putative vault cargo will be an important step towards establishing a function for vaults.

The overall significance of vaults for cellular homeostasis and development was approached by several researchers. In *Dictyostelium*, unlike the situation found in other organisms, three different MVP genes are present, which code for MvpA, MvpB and MvpC of M_r 94 000, 92 000 and 92 000, respectively (Vasu *et al.*, 1993; Vasu and Rome, 1995). Disruption of two (*MvpA* and *MvpB*) of the three MVP genes impedes growth under nutritional stress, suggesting a role for vaults in fundamental processes such as proliferation and cell survival. The relatively mild phenotype is most likely caused by the apparent MVP redundancy in this organism. In mammals, the situation is different as only single genes are coding for the vault proteins. Up till now, two knockout models have been generated in mice in which *TEP1* and *MVP* have been disrupted (Liu *et al.*, 2000; Kickhoefer *et al.*, 2001; Mossink *et al.*, 2002b). In both instances, the mice were healthy, fertile and showed no obvious abnormalities in spite of the absence of distinguishable vault particles in the MVP knockout (Mossink *et al.*, 2002b).

Vaults and multidrug resistance

In 1993, an M_r 110 000 protein was found to be overexpressed in a non-small-cell lung cancer cell line selected for doxorubicin resistance that did not express P-gp (Scheper *et al.*, 1993). This p110 was initially named LRP protein. Screening of an expression library identified LRP as the human MVP (Scheffer *et al.*, 1995), thereby implying a role for vaults in drug resistance. Based on its putative transport function as well as the drug handling and cellular distribution of fluorescent anthracyclines in vault-expressing resistant cell lines, it was proposed that vaults act by transporting drugs away from their subcellular targets by mediating the extrusion of drugs from the nucleus and/or the sequestration of drugs into exocytotic vesicles (see Figure 2 for an overview). In such a scenario, vaults would operate as cytoplasmic and/or nuclear membrane-associated drug transporters perhaps in conjunction with ABC transporters present in the various (intra)cellular membranes. Alternatively, the characteristics and localization of the two minor vault protein, TEP1 and VPARP possibly means that vaults fulfil a role in the protection of the genome and as such contribute to a drug-resistance profile. In the next sections, we will critically review the available experimental evidence concerning vault-mediated drug resistance.

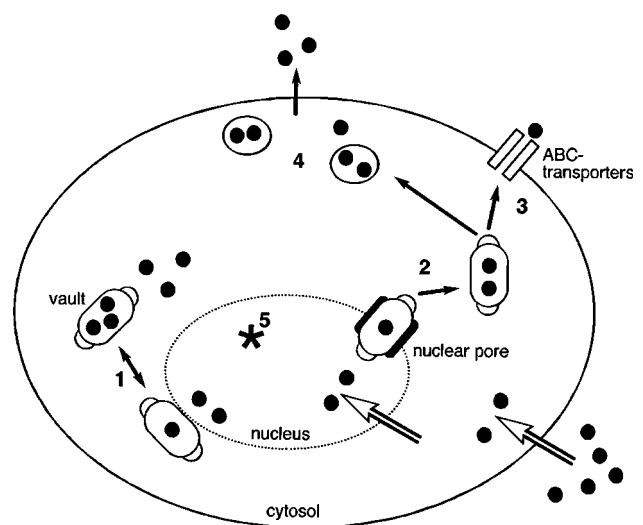


Figure 2 Schematic view of the hypothetical role of vaults in nucleocytoplasmic and vesicular transport of drugs and/or metabolites. Vaults may be involved in the intracellular compartmentalization and/or transport of biomolecules, particularly as it concerns nucleocytoplasmic transport (1 and 2). Vaults may mediate multidrug resistance by transporting drugs away from their intracellular targets, for example, the nucleus (2) or by transporting them to efflux pumps (3) or exocytotic vesicles (4). Based on the characteristics of the minor vault proteins, vaults or vault components are possibly involved in the maintenance of genomic stability, indicated by an asterisk (5). Open arrows represent diffusion and black arrows represent active directional transport. Black dots indicate drugs and biomolecules

***In vitro* studies: MVP transfections, nuclear drug export and knockout models**

Vaults – as judged by the MVP expression – are present in all human tissues, with relatively high levels in cells and tissues that are chronically exposed to xenobiotics, such as lung, epithelial cells in the digestive tract, macrophages and dendritic cells (Kedersha *et al.*, 1990; Izquierdo *et al.*, 1996a; Schroeijers *et al.*, 2002), suggesting a role of vaults in the defense of these organs and cells against toxic compounds. Furthermore, expression of MVP, and the other vault components as well, closely reflected the chemoresistance profile of many tumor cell lines and untreated cancers (Scheper *et al.*, 1993; Izquierdo *et al.*, 1996a, b; Kickhoefer *et al.*, 1998; Schroeijers *et al.*, 2000; Siva *et al.*, 2001). Elevated MVP levels were observed in cell lines resistant to various classes of cytotoxic agents including doxorubicin, mitoxantrone, methotrexate, etoposide, vincristine, cytarabine and cisplatin (Scheper *et al.*, 1993; Versantvoort *et al.*, 1995; Verovski *et al.*, 1996; Laurençot *et al.*, 1997; Moran *et al.*, 1997; Wyler *et al.*, 1997; Komarov *et al.*, 1998). In non-small-cell lung cancer cells, MVP expression levels, determined by protein and mRNA, correlated with resistance to cisplatin (Berger *et al.*, 2000). However, in this study, no correlation was observed with resistance to daunomycin, bleomycin, doxorubicin, etoposide and vinblastine. In contrast, in pharyngeal carcinoma KB-3-1 cells, increased MVP levels were found to correlate with decreased accumulation of doxorubicin in the nuclei of these cells (Cheng *et al.*, 2000). Another recent study performed in U-937 leukemia cells reports that cells selected on doxorubicin upregulated vault levels and acquired resistance against doxorubicin, etoposide and mitoxantrone. This resistance seemed to be independent of P-gp (MDR1), multidrug-resistance-related protein (MRP1), MRP2 and breast cancer resistance protein (BCRP) (Hu *et al.*, 2002).

To assess the role of vaults in drug resistance directly, the ovarian carcinoma cell line A2780 was stably transfected with an MVP expression construct. Although MVP levels were increased, this did not confer drug resistance against doxorubicin, vincristine and etoposide (VP-16) (Scheffer *et al.*, 1995). Initially, this observation was explained by the fact that MVP only comprises 70% of the vault particle mass and that additional factors, that is, the minor vault proteins and/or vRNAs are essential for a proper vault function. However, a recent and more detailed study of the above-mentioned MVP transfectant revealed that these cells do exhibit increased levels of TEPI and VPARP and contain an increased number of intact vault particles (Siva *et al.*, 2001). vRNA levels were not increased, but it is known from previous studies that a pool of vRNA in the cytoplasm exists (Kickhoefer *et al.*, 1998; van Zon *et al.*, 2001) and as such vRNA levels are not limiting for vault formation. The authors conclude that vaults may be necessary but not sufficient for drug resistance. It will be interesting to see these experiments reproduced and extended in a more controlled setting using different cell

lines and transfecting expression constructs for all three vault proteins perhaps in combination with ABC transporters to test the hypothesis whether they work in conjunction.

Recently, the group of Shin-ichi Akiyama reported on experiments supporting a role for vaults of extrusion of anthracyclines from the nuclei of resistant cells (Kitazono *et al.*, 1999, 2001; Ohno *et al.*, 2001). Treatment of the colon carcinoma cell line SW620 with sodium butyrate led to a strong induction of MVP and made the cells significantly less sensitive to doxorubicin, etoposide (VP-16), vincristine, paclitaxel and the transport antibiotic gramicidin D. The stable expression of two unrelated MVP-specific ribozymes reversed the observed resistant phenotype. The molecular mechanism of vault-mediated resistance against doxorubicin was investigated more closely. The drug, which accumulated in the nuclei of untreated cells, was shown to be effluxed more rapidly from the nuclei of sodium butyrate-treated cells. The efflux of doxorubicin, from the nuclei in intact cells or isolated nuclei, could be inhibited by the expression of the ribozymes or the addition of polyclonal anti-(MVP) antibodies (Kitazono *et al.*, 1999). In a subsequent study, the pyridine analog PAK-104P was introduced as specific inhibitor of the vault-mediated efflux (Kitazono *et al.*, 2001). Taken together, these findings provide evidence for the hypothetical model in which vaults function in nuclear drug export and as a consequence may cause drug resistance (see Figure 2). However, it is imperative to further substantiate and verify the model using different drug-resistant, vault-expressing cell lines and/or knockout cell lines in which vaults or vault components are absent. Particularly interesting would be to study the localization and dynamics of the vaults within the nucleus. In addition, more insight is needed in the biochemical requirements of the efflux process, which apparently takes place in a relatively simple buffer without ATP, cytosolic factors, etc. Finally, the sketched molecular mechanism for vault-mediated drug resistance may hold true for anthracyclines and perhaps etoposide, but most likely not for resistance against cytotoxic drugs that target the cytoskeleton like taxol and vincristine.

Exploiting the MVP knockout mouse model, we tested the sensitivity of MVP-deficient cells to a panel of cytostatic agents and found that both embryonic stem cells and bone marrow cells did not show an increased sensitivity to these drugs when compared to wild-type cells (Mossink *et al.*, 2002b). It was shown that the activities of the multidrug-resistance-related transporters P-gp, MRP1 and BCRP1 were not altered in the vault-deficient cells ruling out the possibility that these proteins compensate for the loss of vaults. The *in vivo* toxicity of doxorubicin in MVP knockout mice was also examined. Remarkably, both knockout and control mice responded similarly to the drug treatment. We therefore concluded that – at least in mice – MVP/vaults are not directly involved in drug resistance.

Clinical studies: vaults as a prognostic marker

The hypothesis that MVP expression may reflect a novel pathway of multidrug resistance has prompted several clinical studies to determine the expression of this molecule in human tumors. These studies have mainly focused on the question whether the level of MVP expression predicts the clinical outcome after chemotherapy. The majority of the studies have been performed in hematological malignancies (see Table 2 for an overview), but also other malignancies were examined (see Table 3 for an overview). Various detection techniques of MVP have been used, including immunofluorescence, immunocytochemistry and RNA expression as determined by RT-PCR. The results obtained with these detection assays are variable. Ultimately, a functional assay of MVP activity is needed, since earlier studies with P-glycoprotein (P-gp) have demonstrated the superiority of such an assay for the correlation of *in vitro* drug resistance with clinical response and prognosis. Thus far, the evidence that MVP expression correlates with clinical responses is weak, in particular when small numbers of patients were investigated. Another limitation is that most studies have been founded on univariate analyses without evaluating other prognostic parameters. Thus, compelling evidence that MVP expression correlates with the

clinical response and prognosis is still lacking and should come from a prospective trial using a functional assay and a multivariate analysis of risk factors.

Acute myeloid leukemia

MVP is expressed in 26–91% of patients at diagnosis (Goasguen *et al.*, 1996; List *et al.*, 1996; Hart *et al.*, 1997; Borg *et al.*, 1998; Damiani *et al.*, 1998; Filipits *et al.*, 1998, 2000; Legrand *et al.*, 1998; Leith *et al.*, 1999; Michieli *et al.*, 1999; Pallis *et al.*, 1999; Xu *et al.*, 1999). Unlike P-gp, the expression at relapse or in refractory disease is not elevated as compared to the levels at diagnosis. Several investigators have proposed a negative prognostic significance of MVP expression on the probability to attain a complete response, progression-free survival or relapse-free survival and overall survival (OS) (Goasguen *et al.*, 1996; List *et al.*, 1996; Hart *et al.*, 1997; Borg *et al.*, 1998; Filipits *et al.*, 1998, 2000; Xu *et al.*, 1999). In contrast, other studies did not point towards a prognostic significance of MVP on either outcome variable (Damiani *et al.*, 1998; Legrand *et al.*, 1998; Leith *et al.*, 1999; Michieli *et al.*, 1999; Pallis *et al.*, 1999). In only a few studies, it has been attempted to analyse the prognostic significance of MVP in relation to other drug resistance proteins such as P-gp or the MRP1 protein. In studies searching for coexpression of

Table 2 MVP expression in hematological disorders

Reference	Disease	Patients (n)	MVP detection method	MVP positivity (%)	MVP expression significant prognostic variable for		
					Response to chemotherapy	DFS	OS
Borg <i>et al.</i> (1998)	AML	91	ICC	50	Yes	Yes	Yes
Damiani <i>et al.</i> (1998)	AML	54	FC	61	No	Yes	Yes
Filipits <i>et al.</i> (1998)	AML	86	ICC	36	Yes	No	Yes
Filipits <i>et al.</i> (2000)	AML	111	ICC	34	Yes		
Goasguen <i>et al.</i> (1996)	ALL	20	ICC	35	Yes		No
	AML	26	ICC	69			
Hart <i>et al.</i> (1997)	AML	67	RT-PCR		Yes		
			FC				
Legrand <i>et al.</i> (1998)	AML	47	RT-PCR	70–91	No	No	No
			ICC				
			FC				
Leith <i>et al.</i> (1999)	AML	318	FC	43	No	No	No
List <i>et al.</i> (1996)	AML	77	ICC	33–48	Yes	No	Yes ^b
	CML	10	ICC	10			
Michieli <i>et al.</i> (1999)	AML	96	FC	46	No		
Pallis <i>et al.</i> (1999)	AML	47	FC	26	No		
Xu <i>et al.</i> (1999)	AML	92	RT-PCR		Yes		
den Boer <i>et al.</i> (1998)	AML/ALL	168	FC		Yes ^a		
den Boer <i>et al.</i> (1999)	ALL	85	FC		Yes ^a		
Kakihara <i>et al.</i> (1999)	ALL	40	RT-PCR		No		
Ogretmen <i>et al.</i> (2000)	ALL	14	RT-PCR				
Volm <i>et al.</i> (1997a)	ALL	63	ICC	47–68		Yes	
Ohno <i>et al.</i> (2001)	ATL	38	RT-PCR	68	Yes		Yes
Filipits <i>et al.</i> (1999)	MM	72	ICC	61	Yes		Yes
Raaijmakers <i>et al.</i> (1998)	MM	70	ICC	47	Yes		Yes
Rimza <i>et al.</i> (1999)	MM	73	ICC	74	Yes	No	No

^aMVP expression is significantly related to *in vitro* daunorubicin resistance.

^bBorderline significance $P = 0.058$. Abbreviations: ICC, immunocytochemistry; FC, flow cytometry; RT-PCR, reverse transcriptase-polymerase chain reaction; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; ATL, adult T-cell leukemia; MM, multiple myeloma; DFS, disease free survival; OS, overall survival

Table 3 MVP expression in human cancers

Reference	Disease	Patients (n)	MVP detection method	MVP positivity (%)	MVP expression significant prognostic variable for		
					Response to chemotherapy	DFS	OS
Arts <i>et al.</i> (1999)	Ovarian carcinoma	115	IHC	74	No		No
Izquierdo <i>et al.</i> (1995)	Ovarian carcinoma	57	IHC	77	Yes	Yes	Yes
Linn <i>et al.</i> (1997)	Breast cancer	70	IHC	65	No		
Pohl <i>et al.</i> (1999)	Breast cancer	99	IHC	68	No	No	No
van der Pol <i>et al.</i> (1997)	Melanoma	12	IHC	75	Yes ^a		
Schadendorf <i>et al.</i> (1995)	Melanoma	71	FC				
			IHC	62	Yes ^a		
			FC				
Uozaki <i>et al.</i> (1996)	Osteosarcoma	60	RT-PCR				
			IHC	55	Yes		Yes
Ramani and Dewchand (1995)	Neuroblastoma	21	IHC	57			No
Dingemans <i>et al.</i> (1996)	NSCLC	36	IHC	15–78	No		No
Volm <i>et al.</i> (1997b)	NSCLC	87	IHC	45%	Yes		No

^aData indicate link of MVP expression with drug resistance. Abbreviations: IHC, immunohistochemistry; FC, flow cytometry; RT-PCR, reverse transcriptase-polymerase chain reaction; NSCLC, non-small-cell lung carcinoma; DFS, disease free survival; OS, overall survival

these proteins, simultaneous positivity was observed in 5–24% of cases. In general, the worst response and/or survival was observed in patients who coexpressed P-gp and MVP, while the best prognosis was seen in patients who were negative for both proteins (Goasguen *et al.*, 1996; List *et al.*, 1996; Borg *et al.*, 1998; Filipits *et al.*, 1998). No correlation between MVP expression and other prognostic variables such as FAB classification, older age, high white blood cell count or unfavorable karyotype has been found (Michieli *et al.*, 1997; Legrand *et al.*, 1998; Leith *et al.*, 1999; Van Den Heuvel-Eibrink *et al.*, 2002). From the currently available data it can be concluded that MVP expression is observed in a considerable proportion of the patients, but no conclusive evidence has been found regarding its prognostic significance.

Acute lymphoblastic leukemia (ALL)

Relatively few studies have addressed the role of MVP in ALL. In childhood ALL, a category of ALL with a relatively good prognosis, the proportion of positive patients ranges from 10% at diagnosis up to 68% at relapse (Volm *et al.*, 1997a; den Boer *et al.*, 1998, 1999; Kakiyama *et al.*, 1999). In childhood ALL, reduced intracellular retention of daunorubicin *in vitro* seems to be associated with increased MVP expression, rather than with P-gp or MRP. Moreover, this was associated with a higher level of *in vitro* drug resistance, in general (den Boer *et al.*, 1998, 1999). These data suggest an important role for MVP in the development of resistant disease in ALL. Another group pointed to the higher incidence of MVP expression at relapse (68%) as compared to diagnosis (47%), which suggests that MVP expression can either be induced during chemotherapy or is selected for through prior treatment (Volm *et al.*, 1997a). Again, there seems to be no significant correlation with other prognostic parameters in ALL. MVP expression may be higher in leukemias with a pre-B-cell origin as compared to T-cell ALL.

None of these studies seriously addressed the prognostic impact of MVP expression on the outcome of clinical chemotherapy, primarily because of the small size of the patient groups (Volm *et al.*, 1997a; Kakiyama *et al.*, 1999; Ogretmen *et al.*, 2000). Currently, the role of MVP expression is prospectively evaluated in several clinical studies. In adult T-cell leukemia, which is a disease more common in Asia and caused by the HTLV-1 virus, MVP expression is high and is considered to be a negative prognostic factor for OS and response to chemotherapy (Ohno *et al.*, 2001).

Multiple myeloma (MM)

In MM, expression of MVP is observed in 47–74% of untreated patients (Raaijmakers *et al.*, 1998; Filipits *et al.*, 1999; Rimsza *et al.*, 1999). Three studies have addressed the prognostic significance of the protein using immunocytochemistry. In all studies, MVP expression is associated with relative resistance to standard treatment with melphalan-based regimens. However, this relationship was found only in patients who were treated with a conventional dose of melphalan and not in patients receiving escalating dosages (Raaijmakers *et al.*, 1998). Taken together, the observations in MM warrant further investigations into the role of MVP in drug resistance in MM.

Solid tumors

Relatively few studies have addressed the role of MVP in solid tumors. Two studies from the same group have investigated the expression of MVP in ovarian cancer (Izquierdo *et al.*, 1995; Arts *et al.*, 1999). In advanced ovarian cancer FIGO stage III/IV, 77% of the patients express MVP at diagnosis. In localized cancer FIGO stage I/II, a similar figure is observed. In advanced ovarian cancer, a correlation was found between MVP expression and lack of response and/or shorter OS. This was not found in early-stage ovarian cancer. In contrast,

in early-stage ovarian cancer, MVP expression was associated with favorable prognostic variables.

Two studies were performed in breast cancer, which both used immunohistochemistry to investigate MVP expression. In one study, the expression ranged from 69 to 75% without significant differences between samples obtained at diagnosis or at relapse after chemotherapy (Linn *et al.*, 1997). The second study found 68% of patients with intermediate or high MVP expression (Pohl *et al.*, 1999). In neither study, a clear correlation with clinical outcome was observed.

Other tissues that express a high level of MVP include bronchial epithelium, cecum/rectum, colon and other epithelial tissues. In cancers derived from these tissues, a variable expression of MVP is observed. For example, in non-small-cell and small-cell lung cancer, the expression is different with the highest expression found in chemoresistant non-small-cell lung cancer (Dingemans *et al.*, 1996). As in another study, no correlation with relevant clinical or clinicopathological parameters was observed (Dingemans *et al.*, 1996; Volm *et al.*, 1997b). The strongest expression of MVP is found in colorectal tumors. In this tumor, the expression increases from premalignant lesions such as colonic adenoma to aggressive colon carcinoma, which indicates that MVP may be associated with more aggressive disease (Izquierdo *et al.*, 1996a; Meijer *et al.*, 1999). Other tumors in which expression of MVP has been reported include melanoma, osteosarcoma and neuroblastoma (Ramani and Dewchand, 1995). In melanoma, a high expression is observed, which seems to correlate with aggressive behavior of the tumor, such as in primary choroidal melanoma (Schadendorf *et al.*, 1995; van der Pol *et al.*, 1997). Since this tumor type rarely responds to chemotherapy, the relevance for drug resistance remains unclear. In osteosarcoma, MVP expression was reported to correlate with failure to chemotherapy and poor survival (Uozaki *et al.*, 1997).

Conclusions/future prospects

It is evident that MVP/vaults are somehow associated with chemoresistance in primary tumors and various tumor cell lines. In addition, several clinical studies – but not all – do recognize MVP as a negative prognostic factor for response to chemotherapy and/or disease-free survival (DFS) and/or OS. The main question is, however, whether vaults themselves play a direct role

in drug resistance or whether they have to be merely considered as a marker of a drug resistance phenotype. Up till now, few studies have attempted to determine the contribution of vaults to drug resistance. In murine cells, the absence of vaults does not give rise to a hypersensitivity for drugs (Mossink *et al.*, 2002b), whereas in MVP/vaults-overexpressing colon carcinoma cells anthracyclines are cleared from the nucleus in an MVP-dependent fashion (Kitazono *et al.*, 1999, 2001).

It is clear that additional studies employing similar and validated techniques in different tumor samples are needed to determine unequivocally whether the vaults expression has prognostic significance. Furthermore, future experiments should address whether intact vaults are capable of binding and transporting drugs, determine whether vaults associate and copurify with exocytotic vesicles and more detailed studies are needed into the subcellular localization and dynamics of the vault complex. In general, more insight is needed into the normal cellular function of vaults and its relation to the nonvault-associated pools of VPARP and TEPI. Intriguing research leads are the identification of the putative vault cargo, of additional VPARP substrates, the elucidation of the role VPARP plays in the nucleus and the determination of the conditions that induce VPARP activity. Equally interesting are more detailed studies into a possible crosstalk and cooperation between vaults and the telomerase complex, particularly, since it was recently published that both VPARP and vRNA are also partially associated with the telomerase complex (Shall, 2002). In all these studies, the available *TEPI* and *MVP* knockout models will be highly instrumental and most certainly be essential to reveal the full significance of vaults.

Abbreviations

MVP, major vault protein; TEPI, telomerase-associated protein; VPARP, vault poly-(ADP-ribose) polymerase; vRNA, vault RNA; LRP, lung resistance-related protein; P-gp, P-glycoprotein; MRP1, multidrug-resistance-related protein; BCRP, breast cancer resistance protein.

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References

- Abbondanza C, Rossi V, Roscigno A, Gallo L, Belsito A, Piluso G, Medici N, Nigro V, Molinari AM, Moncharmont B and Puca GA. (1998). *J. Cell Biol.*, **141**, 1301–1310.
- Arts HJ, Katsaros D, de Vries EG, Massobrio M, Genta F, Danese S, Arisio R, Scheper RJ, Kool M, Scheffer GL, Willemsse PH, van der Zee AG and Suurmeijer AJ. (1999). *Clin. Cancer Res.*, **5**, 2798–2805.
- Beattie TL, Zhou W, Robinson MO and Harrington L. (1998). *Curr. Biol.*, **8**, 177–180.
- Berger W, Elbling L and Micksche M. (2000). *Int. J. Cancer*, **88**, 293–300.
- Borg AG, Burgess R, Green LM, Scheper RJ and Yin JA. (1998). *Br. J. Haematol.*, **103**, 1083–1091.
- Cheng SH, Lam W, Lee AS, Fung KP, Wu RS and Fong WF. (2000). *Toxicol. Appl. Pharmacol.*, **164**, 134–142.
- Chugani DC, Rome LH and Kedersha NL. (1993). *J. Cell Sci.*, **106**, 23–29.

- Damiani D, Michieli M, Ermacora A, Candoni A, Raspadori D, Geromin A, Stocchi R, Grimaz S, Masolini P, Michelutti A, Scheper RJ and Baccarani M. (1998). *Haematologica*, **83**, 290–297.
- den Boer ML, Pieters R, Kazemier KM, Janka-Schaub GE, Henze G and Veerman AJ. (1999). *Leukemia*, **13**, 2023–2030.
- den Boer ML, Pieters R, Kazemier KM, Rottier MM, Zwaan CM, Kaspers GJ, Janka-Schaub G, Henze G, Creutzig U, Scheper RJ and Veerman AJ. (1998). *Blood*, **91**, 2092–2098.
- Dingemans AM, van Ark-Otte J, van der Valk P, Apolinario RM, Scheper RJ, Postmus PE and Giaccone G. (1996). *Ann. Oncol.*, **7**, 625–630.
- Filipits M, Drach J, Pohl G, Schuster J, Stranzl T, Ackermann J, Konigsberg R, Kaufmann H, Gisslinger H, Huber H, Ludwig H and Pirker R. (1999). *Clin. Cancer Res.*, **5**, 2426–2430.
- Filipits M, Pohl G, Stranzl T, Suchomel RW, Scheper RJ, Jager U, Geissler K, Lechner K and Pirker R. (1998). *Blood*, **91**, 1508–1513.
- Filipits M, Stranzl T, Pohl G, Heinzl H, Jäger U, Geissler K, Fonatsch C, Haas OA, Lechner K and Pirker R. (2000). *Leukemia*, **14**, 68–76.
- Goasguen JE, Lamy T, Bergeron C, Ly Sunaram B, Mordelet E, Gorre G, Dossot JM, Le Gall E, Grosbois B, Le Prise PY and Fauchet R. (1996). *Leukemia Lymphoma*, **23**, 567–576.
- Hamill DR and Suprenant KA. (1997). *Dev. Biol.*, **190**, 117–128.
- Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Bass MB, Arruda I and Robinson MO. (1997). *Science*, **275**, 973–977.
- Hart SM, Ganeshaguru K, Scheper RJ, Prentice HG, Hoffbrand AV and Mehta AB. (1997). *Exp. Hematol.*, **25**, 1227–1232.
- Herrmann C, Golkaramnay E, Inman E, Rome L and Volkandt W. (1999). *J. Cell Biol.*, **144**, 1163–1172.
- Hu Y, Stephen AG, Cao J, Tanzer LR, Slapak CA, Harrison SD, Devanarayan V, Dantzig AH, Starling JJ, Rome LH and Moore RE. (2002). *Int. J. Cancer*, **97**, 149–156.
- Izquierdo MA, Scheffer GL, Flens MJ, Giaccone G, Broxterman HJ, Meijer CJ, van der Valk P and Scheper RJ. (1996a). *Am. J. Pathol.*, **148**, 877–887.
- Izquierdo MA, Shoemaker RH, Flens MJ, Scheffer GL, Wu L, Prather TR and Scheper RJ. (1996b). *Int. J. Cancer*, **65**, 230–237.
- Izquierdo MA, van der Zee AG, Vermorken JB, van der Valk P, Belien JA, Giaccone G, Scheffer GL, Flens MJ, Pinedo HM, Kenemans P, Meijer CJ, de Vries EG and Scheper RJ. (1995). *J. Natl. Cancer Inst.*, **87**, 1230–1237.
- Johansson M. (1999). *Genomics*, **57**, 442–445.
- Kakihara T, Tanaka A, Watanabe A, Yamamoto K, Kanto K, Kataoka S, Ogawa A, Asami K and Uchiyama M. (1999). *Pediatr. Int.*, **41**, 641–647.
- Kedersha NL, Heuser JE, Chugani DC and Rome LH. (1991). *J. Cell Biol.*, **112**, 225–235.
- Kedersha NL, Miquel MC, Bittner D and Rome LH. (1990). *J. Cell Biol.*, **110**, 895–901.
- Kedersha NL and Rome LH. (1986). *J. Cell Biol.*, **103**, 699–709.
- Kedersha NL and Rome LH. (1990). *Mol. Biol. Rep.*, **14**, 121–122.
- Kickhoefer VA, Liu Y, Kong LB, Snow BE, Stewart PL, Harrington L and Rome LH. (2001). *J. Cell Biol.*, **152**, 157–164.
- Kickhoefer VA, Rajavel KS, Scheffer GL, Dalton WS, Scheper RJ and Rome LH. (1998). *J. Biol. Chem.*, **273**, 8971–8974.
- Kickhoefer VA, Searles RP, Kedersha NL, Garber ME, Johnson DL and Rome LH. (1993). *J. Biol. Chem.*, **268**, 7868–7873.
- Kickhoefer VA, Siva AC, Kedersha NL, Inman EM, Ruland C, Streuli M and Rome LH. (1999a). *J. Cell Biol.*, **146**, 917–928.
- Kickhoefer VA, Stephen AG, Harrington L, Robinson MO and Rome LH. (1999b). *J. Biol. Chem.*, **274**, 32712–32717.
- Kickhoefer VA, Vasu SK and Rome LH. (1996). *Trends Cell Biol.*, **6**, 174–178.
- Kitazono M, Okumura H, Ikeda R, Sumizawa T, Furukawa T, Nagayama S, Seto K, Aikou T and Akiyama S. (2001). *Int. J. Cancer*, **91**, 126–131.
- Kitazono M, Sumizawa T, Takebayashi Y, Chen ZS, Furukawa T, Nagayama S, Tani A, Takao S, Aikou T and Akiyama S. (1999). *J. Natl. Cancer Inst.*, **91**, 1647–1653.
- Komarov PG, Shtil AA, Holian O, Tee L, Buckingham L, Mechetner EB, Roninson IB and Coon JS. (1998). *Oncol. Res.*, **10**, 185–192.
- Kong LB, Siva AC, Kickhoefer VA, Rome LH and Stewart PL. (2000). *RNA*, **6**, 890–900.
- Kong LB, Siva AC, Rome LH and Stewart PL. (1999). *Struct. Fold Des.*, **7**, 371–379.
- Lange C, Walther W, Schwabe H and Stein U. (2000). *Biochem. Biophys. Res. Commun.*, **278**, 125–133.
- Laurençot CM, Scheffer GL, Scheper RJ and Shoemaker RH. (1997). *Int. J. Cancer*, **72**, 1021–1026.
- Legrand O, Simonin G, Zittoun R and Marie JP. (1998). *Leukemia*, **12**, 1367–1374.
- Leith CP, Kopecky KJ, Chen IM, Eijdens L, Slovak ML, McConnell TS, Head DR, Weick J, Grever MR, Appelbaum FR and Willman CL. (1999). *Blood*, **94**, 1086–1099.
- Li JY, Volkandt W, Dahlstrom A, Herrmann C, Blasi J, Das B and Zimmermann H. (1999). *Neuroscience*, **91**, 1055–1065.
- Linn SC, Pinedo HM, van Ark-Otte J, van der Valk P, Hoekman K, Honkoop AH, Vermorken JB and Giaccone G. (1997). *Int. J. Cancer*, **71**, 787–795.
- List AF, Spier CS, Grogan TM, Johnson C, Roe DJ, Greer JP, Wolff SN, Broxterman HJ, Scheffer GL, Scheper RJ and Dalton WS. (1996). *Blood*, **87**, 2464–2469.
- Liu Y, Snow BE, Hande MP, Baerlocher G, Kickhoefer VA, Yeung D, Wakeham A, Itie A, Siderovski DP, Lansdorp PM, Robinson MO and Harrington L. (2000). *Mol. Cell Biol.*, **20**, 8178–8184.
- Ma Q, Baldwin KT, Renzelli AJ, McDaniel A and Dong L. (2001). *Biochem. Biophys. Res. Commun.*, **289**, 499–506.
- Masson M, Niedergang C, Schreiber V, Muller S, Menissier-de Murcia J and de Murcia G. (1998). *Mol. Cell Biol.*, **18**, 3563–3571.
- Meijer GA, Schroeijers AB, Flens MJ, Meuwissen SG, van der Valk P, Baak JP and Scheper RJ. (1999). *J. Clin. Pathol.*, **52**, 450–454.
- Michieli M, Damiani D, Ermacora A, Masolini P, Raspadori D, Visani G, Scheper RJ and Baccarani M. (1999). *Br. J. Haematol.*, **104**, 328–335.
- Michieli M, Damiani D, Ermacora A, Raspadori D, Michelutti A, Grimaz S, Fanin R, Russo D, Lauria F, Masolini P and Baccarani M. (1997). *Br. J. Haematol.*, **96**, 356–365.
- Moran E, Cleary I, Larkin AM, Amhloibh RN, Masterson A, Scheper RJ, Izquierdo MA, Center M, O'Sullivan F and Clynes M. (1997). *Eur. J. Cancer*, **33**, 652–660.
- Mossink MH, van Zon A, Fränzel-Luiten E, Schoester M, Kickhoefer VA, Scheffer GL, Scheper RJ, Sonneveld P and Wiemer EAC. (2002b). *Cancer Res.*, **62**, 7298–7304.

- Mossink MH, van Zon A, Fränzel-Luiten E, Schoester M, Scheffer GL, Scheper RJ, Sonneveld P and Wiemer EA. (2002a). *Gene*, **294**, 225–232.
- Ogretmen B, Barredo JC and Safa AR. (2000). *J. Pediatr. Hematol. Oncol.*, **22**, 45–49.
- Ohno N, Tani A, Uozumi K, Hanada S, Furukawa T, Akiba S, Sumizawa T, Utsunomiya A, Arima T and Akiyama S. (2001). *Blood*, **98**, 1160–1165.
- Oliver FJ, Menissier-de Murcia J and de Murcia G. (1999). *Am. J. Hum. Genet.*, **64**, 1282–1288.
- Pallis M, Turzanski J, Harrison G, Wheatley K, Langabeer S, Burnett AK and Russell NH. (1999). *Br. J. Haematol.*, **104**, 307–312.
- Pohl G, Filipits M, Suchomel RW, Stranzl T, Depisch D and Pirker R. (1999). *Anticancer Res.*, **19**, 5051–5055.
- Raaijmakers HG, Izquierdo MA, Lokhorst HM, de Leeuw C, Belien JA, Bloem AC, Dekker AW, Scheper RJ and Sonneveld P. (1998). *Blood*, **91**, 1029–1036.
- Ramani P and Dewchand H. (1995). *J. Pathol.*, **175**, 13–22.
- Rimsza LM, Campbell K, Dalton WS, Salmon S, Willcox G and Grogan TM. (1999). *Leukemia Lymphoma*, **34**, 315–324.
- Rome LH, Kedersha NL and Chugani DC. (1991). *Trends Cell Biol.*, **1**, 47–50.
- Sbodio JI, Lodish HF and Chi NW. (2002). *Biochem. J.*, **361**, 451–459.
- Schadendorf D, Makki A, Stahr C, van Dyck A, Wanner R, Scheffer GL, Flens MJ, Scheper R and Henz BM. (1995). *Am. J. Pathol.*, **147**, 1545–1552.
- Scheffer GL, Wijngaard PL, Flens MJ, Izquierdo MA, Slovak ML, Pinedo HM, Meijer CJ, Clevers HC and Scheper RJ. (1995). *Nat. Med.*, **1**, 578–582.
- Scheper RJ, Broxterman HJ, Scheffer GL, Kaaijk P, Dalton WS, van Heijningen TH, van Kalken CK, Slovak ML, de Vries EG, van der Valk P, Meijer CJ and Pinedo HM. (1993). *Cancer Res.*, **53**, 1475–1479.
- Schreiber V, Ame JC, Dolle P, Schultz I, Rinaldi B, Fraulob V, Menissier-De Murcia J and de Murcia G. (2002). *J. Biol. Chem.*, **277**, 23028–23036.
- Schroeijers AB, Reurs AW, Scheffer GL, Stam AG, de Jong MC, Rustemeyer T, Wiemer EA, de Gruijl TD and Scheper RJ. (2002). *J. Immunol.*, **168**, 1572–1578.
- Schroeijers AB, Siva AC, Scheffer GL, de Jong MC, Bolick SC, Dukers DF, Sloodstra JW, Meloen RH, Wiemer E, Kickhoefer VA, Rome LH and Scheper RJ. (2000). *Cancer Res.*, **60**, 1104–1110.
- Shall S. (2002). *BioEssays*, **24**, 197–201.
- Siva AC, Raval-Fernandes S, Stephen AG, LaFemina MJ, Scheper RJ, Kickhoefer VA and Rome LH. (2001). *Int. J. Cancer*, **92**, 195–202.
- Smith S. (2001). *Trends Biochem. Sci.*, **26**, 174–179.
- Smith S, Giriati I, Schmitt A and de Lange T. (1998). *Science*, **282**, 1484–1487.
- Stephen AG, Raval-Fernandes S, Huynh T, Torres M, Kickhoefer VA and Rome LH. (2001). *J. Biol. Chem.*, **276**, 23217–23220.
- Stoffler D, Goldie KN, Feja B and Aebi U. (1999). *J. Mol. Biol.*, **287**, 741–752.
- Uozaki H, Horiuchi H, Ishida T, Iijima T, Imamura T and Machinami R. (1997). *Cancer*, **79**, 2336–2344.
- Van Den Heuvel-Eibrink MM, Wiemer EA, Prins A, Meijerink JP, Vosseveld PJ, Van Der Holt B, Pieters R and Sonneveld P. (2002). *Leukemia*, **16**, 833–839.
- van der Pol JP, Blom DJ, Flens MJ, Luyten GP, de Waard-Siebinga I, Koornneef L, Scheper RJ and Jager MJ. (1997). *Invest. Ophthalmol. Vis. Sci.*, **38**, 2523–2530.
- van Zon A, Mossink MH, Schoester M, Scheffer GL, Scheper RJ, Sonneveld P and Wiemer EA. (2001). *J. Biol. Chem.*, **276**, 37715–37721.
- van Zon A, Mossink MH, Schoester M, Scheffer GL, Scheper RJ, Sonneveld P and Wiemer EA. (2002). *Biochem. Biophys. Res. Commun.*, **291**, 535–541.
- Vasu SK, Kedersha NL and Rome LH. (1993). *J. Biol. Chem.*, **268**, 15356–15360.
- Vasu SK and Rome LH. (1995). *J. Biol. Chem.*, **270**, 16588–16594.
- Verovski VN, Van den Berge DL, Delvaeye MM, Scheper RJ, De Neve WJ and Storme GA. (1996). *Br. J. Cancer*, **73**, 596–602.
- Versantvoort CH, Withoff S, Broxterman HJ, Kuiper CM, Scheper RJ, Mulder NH and de Vries EG. (1995). *Int. J. Cancer*, **61**, 375–380.
- Volm M, Mattern J and Koomagi R. (1997b). *Anticancer Drugs*, **8**, 931–936.
- Volm M, Stammer G, Zintl F, Koomagi R and Sauerbrey A. (1997a). *Anticancer Drugs*, **8**, 662–665.
- Weinrich SL, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtsteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB and Morin GB. (1997). *Nat. Genet.*, **17**, 498–502.
- Wylter B, Shao Y, Schneider E, Cianfriglia M, Scheper RJ, Frey BM, Gieseler F, Schmid L, Twentyman PR and Lehnert M. (1997). *Br. J. Haematol.*, **97**, 65–75.
- Xu D, Arestrom I, Virtala R, Pisa P, Peterson C and Gruber A. (1999). *Br. J. Haematol.*, **106**, 627–633.
- Yu Z, Fotouhi-Ardakani N, Wu L, Maoui M, Wang S, Banville D and Shen S-H. (2002). *J. Biol. Chem.*, **277**, 40247–40252.