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Multidrug Resistance and its Circumvention

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MULTIDRUG RESISTANCE (MDR) is now a common and well-studied experimental phenomenon [1–3] that appears to have clinical correlates [4] and may play a role in clinical resistance to antineoplastic agents [5]. By now, the basic features of MDR are well known. Mammalian cells selected for resistance to a 'natural product' anticancer drug display cross-resistance to a variety of other agents that have no apparent commonality, save that they are in general rather large (M_r ranging from ~300 to 900), lipophilic, and do not appear to enter cells by specific carrier proteins. Such cells accumulate and retain less drug than do their drug-sensitive counterparts, and this is presumably the cellular basis for their resistance [6]. This alteration in cellular pharmacology is likely mediated by P-glycoprotein (Pgp), a large (M_r ~ 170,000) integral membrane protein that is thought to affect the rapid efflux of drugs from the cell [1–3]. The cDNA encoding the protein has been cloned and sequenced [7–9], inserted into an expression vector, and transfected into drug-sensitive cells with the result that the transfectants express the full MDR phenotype [10, 11]. These types of experiments support the hypothesis that Pgp functions to export these large, lipophilic cytotoxic agents from the cell. Based on its normal expression in colon, small intestine, adrenal, kidney and hepatic tissues [12, 13], it has been proposed [14] that Pgp plays a role in the excretion of xenobiotics from the body.

Recent descriptions of MDR *not* associated with Pgp overexpression have added a degree of complexity to the definitions of MDR. For example, several anthracycline-resistant cell lines have been established that exhibit drug transport defects but do not appear to express Pgp [15–17]. The basis for this resistance is not clear, but it may be related to alterations in intracelllular drug distribution [15]. Furthermore, we and others have described cell lines expressing a form of MDR associated with decreases in the catalytic activity of and DNA cleavage by the essential nuclear enzyme, topoisomerase II [18–21]. Cells expressing this type of MDR, originally termed by us 'atypical' MDR (at-MDR), do not overexpress Pgp, and are unaltered in their ability to accumulate or retain drugs, compared to drug-sensitive cells. I will confine my remarks here to mechanisms of Pgp-associated MDR (Pgp-MDR).

DRUG ACCUMULATION AND RETENTION

Insights into the mechanism by which Pgp affects drug accumulation and retention come from experiments showing that a photoactive azido analog of vinblastine, a drug to which Pgp-MDR cells express resistance and cross-resistance, specifically binds to Pgp [22]. This binding can be competed to varying degrees by other 'MDR-type' drugs (vincristine, doxorubicin, etc.) as well as by 'modulator' compounds (verapamil, reserpine, etc.) [23] that can overcome this resistance by enhancing cellular levels of anticancer drug, thereby allowing more of the agent to reach its cytotoxic target(s). Despite earlier interpretations suggesting that there was little specific structural similarity among drugs or modulators associated with the Pgp-MDR phenotype [6], it now appears that these anticancer drugs and most Pgp-MDR modulators are recognized and bound by Pgp [22, 23].

MECHANISMS OF CYTOTOXICITY AND RESISTANCE

Regardless of their diverse mechanism(s) of cytotoxicity, it is clear that many 'natural product' compounds share at least one common mechanism of resistance—that mediated by Pgp. Thus, *Vinca* alkaloids (whose cytotoxicity is mediated through binding to tubulin), epipodophyllotoxins and aminoacridines (which inhibit DNA topoisomerase II activity), and anthracyclines (which appear to have membrane, cytoplasmic, and nuclear cytotoxic targets) all exhibit diminished effectiveness in cells overexpressing Pgp. More recent findings indicate that compounds that target mitochondria also share this Pgp mechanism of resistance. For example, Pgp-MDR cells are cross-resistant to the mitochondrial-specific dye, rhodamine 123 [24], as well as the quaternized bis-quinolinium phthalanilide analog, QBQ, described by Liley *et al.* [25]. The apparent mitochondrial targeting of these compounds, however, is probably irrelevant in terms of resistance mechanisms. That Pgp-MDR cells are cross-resistant to these compounds is likely due to the very real possibility that they serve as substrates for Pgp; indeed, this appears to be the case for rhodamine 123 [24, 26]. It will be of considerable interest to determine whether QBQ competes with a photoaffinity analog of vinblastine for binding to Pgp.

Pgp-MDR 'MODULATORS'

Studies of modulators of Pgp-MDR have provided some insights into both the mechanism(s) of and the direction for subsequent studies of this form of MDR. For example, recent work suggests that there are indeed structural requirements for a modulator to bind to Pgp [23, 27]. My colleagues and I proposed some 'rules' for a compound to be a good modulator of MDR—it should be hydrophobic, have two aromatic rings and a positively charged nitrogen [28]. Cationic, lipophilic molecules such as the mitochondrion-targeting agents, rhodamine 123 and QBQ, would appear to fulfill these criteria. Other agents such as cyclosporin [29], tamoxifen [30] and Tween 80 [31] also modulate Pgp-MDR, but, with the possible exception of tamoxifen, they do not appear to share all of these characteristics. These modulators, however, are all highly lipophilic, suggesting

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that this may be the most important feature of a modulating agent. Likewise, while many of the anticancer drugs involved in Pgp-MDR share some or all of these characteristics, others do not (the anthracylines are less hydrophobic and the epipodophyllotoxins are not charged), supporting the suggestion that other factors may be important in drug recognition by Pgp.

Many Pgp-MDR modulators belong to the drug classes of calcium channel blockers and calmodulin inhibitors, the prototypes being verapamil and trifluoperazine, respectively. However, Pgp-MDR cells have neither voltage gated calcium channels [32] nor calmodulin levels that differ from those of the drug-sensitive parent cells [33]. It is clear that many of these modulators exert their action by competing with the anti-cancer drugs for binding to Pgp [22, 23, 27] and in fact bind to Pgp themselves [34, 35]. These compounds, however, are not cytotoxic to the Pgp-MDR cells at the concentrations used. Why, then, are these classes of modulators so effective in binding to Pgp and in 'reversing' MDR? The binding data suggest that whatever binding pocket in Pgp is recognized by the 'Ca^{2+} channel blockers', it must resemble that of the calcium channel in excitable tissue. This idea is supported by the recent cloning of bovine brain adenylyl cyclase, which was found to have a striking topographical resemblance to Pgp [36].

SUBCELLULAR DRUG DISTRIBUTION

The subcellular distribution of drugs in MDR cells has not been studied extensively. Anthracyclines have been shown to distribute in punctate cytoplasmic structures in MDR cells, whereas these drugs exhibit a more diffuse nuclear fluorescence in their drug-sensitive counterparts. While these punctate structures were not identified functionally, it was suggested [1,15, 37] that they might be acidic compartments, possibly endosomes or lysosomes. These results may need to be re-evaluated in light of the results with rhodamine 123 [24] and QBQ [25]. One would have liked to have seen comparisons of QBQ distribution in drug-sensitive and -resistant cells as well as functional biochemical identification of these structures in the paper by Liley *et al.* [25]. Whatever these structures represent, however, it is not clear why drugs such as anthracyclines distribute differently in Pgp-MDR cells than in drug-sensitive cells. These observations suggest that the altered subcellular drug distribution may be important in the expression of the Pgp-MDR phenotype, and that these subcellular structures may sequester any accumulated drug into metabolically inert compartments [1].

Pgp-MDR MODULATORS AND SUBCELLULAR DRUG DISTRIBUTION

Modulators of Pgp-MDR alter the cytoplasmic distribution of drugs in these cells. For example, Chauffert *et al.* showed [38] that doxorubicin was accumulated first by nuclei of MDR colon carcinoma cells and with time redistributed to discrete cytoplasmic structures; verapamil prevented this redistribution. More recently, Hindenburg *et al.* showed a similar effect in anthracycline-resistant HL-60 cells [15]. However, since those MDR HL-60 cells do not express measurable amounts of *mdr1* mRNA or Pgp [39], the effect of verapamil on intracellular drug redistribution and drug efflux via Pgp may not be functionally linked. It remains to be demonstrated whether modulators such as verapamil affect the subcellular distribution of cationic lipophilic compounds like rhodamine 123 and QBQ that primarily target the mitochondria. Likewise, it will be of considerable interest to determine whether other modulators have the ability to alter the subcellular distribution of anticancer drugs such as doxorubicin.

MEMBRANE POTENTIALS IN Pgp-MDR

The apparent mitochondrial accumulation of rhodamine 123 [26, 40] and QBQ [25] can be altered by conditions that dissipate either the plasma membrane or mitochondrial membrane potentials. The role of these membrane potentials in Pgp-MDR has only recently been studied and shown to differ between the drug-resistant and -sensitive cells [41, 42]. Importantly, both verapamil [41, 42] and cyclosporin [41] have been shown to correct the decreased membrane potentials of Pgp-MDR cells. Verapamil also enhances ATP consumption [43], and decreases the increased cytoplasmic pH of Pgp-MDR cells [44]. The relationship among membrane potential, ATP consumption and cytosolic pH is presently unclear, but it is possible that increasing ATP consumption could affect the other two. Clearly, more investigations of these functions in cells expressing Pgp-MDR are warranted. Also, since the cationic dyes used to determine membrane potential are themselves likely to be substrates for Pgp [24, 26], some assessment of membrane potential independent of dye accumulation would be desirable.

EFFECT OF Pgp ON TUMOR CELL PHYSIOLOGY

Abundant amounts of Pgp in the plasma membrane are likely to affect cellular physiology and homeostasis. It is known that plasma membrane lipid fluidity is in general increased in cells overexpressing Pgp [1, 45], although one report suggests the opposite [46]. As fluid-phase endocytosis is increased in Pgp-MDR cell lines [47], Pgp may destabilize the plasma membrane [1], thus increasing membrane 'trafficking'. The consequences of this might be increased cytoplasmic pH in Pgp-MDR cell lines, as recently reported [44], suggesting that the Na^+-H^+ antiporter might be affected by Pgp either directly or indirectly through, e.g., increased membrane turnover. Whether the effect of verapamil to decrease cytoplasmic pH toward that of drug-sensitive cells [44] is related to its ability to alter subcellular drug distribution [38] or to its effect on ATP utilization [43] remains to be determined.

CONCLUSION

Much is now known about Pgp-MDR and compounds that modulate it. Less clear, however, is the mechanism by which these compounds interact with Pgp. Also unclear is the significance of the altered subcellular distribution of drugs in Pgp-MDR cells. Moreover, the role of plasma membrane and mitochondrial membrane potentials in Pgp-MDR needs further examination. Detailed structure–activity studies, such as those now being done with modulators of Pgp-MDR [23], as well as studies with new classes of compounds [25], may provide insights into the mechanisms of Pgp-MDR and direction for the design of new therapeutic agents.

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