The extended-MDR phenotype

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Abstract

Cellular models have made a significant contribution to our understanding of the molecular mechanisms of resistance to chemotherapeutic drugs. However the vast majority of these models involve cell sublines with high levels of resistance generated by continuous exposure to high drug doses, and although the majority express a multidrug resistance (MDR) phenotype, they fall short of the broader drug cross resistance that is characteristic of cancers which no longer respond to treatment. Several studies have reported cell sublines which not only have the MDR phenotype and are resistant to 'natural product' lipophilic drugs, but they are also resistant to alkylating agents and antimetabolites. A common feature of these sublines is they were generated by treatment with low, clinically relevant levels of drug given intermittently. The term extended-MDR has been used to describe this type of broad drug cross resistance. Here we review those factors that promote the development of extended-MDR, the characteristics of extended-MDR sublines and the possible resistance mechanisms involved.

Introduction

Half of all patients with cancer are cured. The half that are not cured either have cancers that are inherently drug resistant or they have developed drug resistance in response to treatment. Drug resistance remains the main problem in the treatment of cancer. The clinical reality is that a cancer which shows a reduced response will progress to being resistant to all chemotherapy and radiotherapy treatment. The spectrum of drugs involved in this resistance is therefore very broad.

Cellular models of drug resistance have defined possible molecular mechanisms of drug resistance and these have been reviewed in other chapters. Typically these cellular models are obtained by culturing cancer cells continuously in the presence of increasing concentrations of a single chemotherapeutic drug and this usually produces drug resistant sublines that can grow in the presence of high concentrations of the selecting drug. In some studies a series of sublines are developed with increasing levels of resistance. How-

ever it is usually the highly drug resistant sublines that are most studied and the majority of drug resistance mechanisms described have been from highly drug resistant sublines grown continuously in the presence of the selecting drug.

When the selecting drug is a 'natural product' lipophilic drug such as an anthracycline, *Vinca* alkaloid, epipodophyllotoxin or taxane, then MDR usually develops. The resistance mechanism most likely to cause MDR in the highly drug resistant sublines is the expression of the *MDR1* gene product, P-glycoprotein (Endicott and Ling, 1989; Gottesman *et al.*, 1995). P-glycoprotein is a 170 kDa transmembrane glycoprotein and is a member of the ABC superfamily of ATP-dependent transporters. Cells expressing P-glycoprotein are resistant to natural product lipophilic drugs. Other resistance mechanisms resulting from selection with natural product lipophilic drugs include the overexpression of the multidrug resistance-associated protein, MRP (Cole *et al.*, 1992; Loe *et al.*, 1996; Kavallaris, 1997) which is a 190 kDa trans-

membrane glycoprotein also belonging to the ABC superfamily. Cells overexpressing MRP are resistant to natural product lipophilic drugs with the notable exception of paclitaxel (Davey *et al.*, 1997; Kavalakis, 1997). There are also many examples of drug resistant sublines with altered glutathione metabolism (O'Brien and Tew, 1996) and/or altered topoisomerase II (Boege, 1996) that have resulted from this type of drug selection.

Similar treatment of cells with platinum-containing drugs or other alkylating agents usually produces drug resistant sublines with altered glutathione metabolism (Tew, 1994) and/or altered DNA repair (Chao, 1996). Decreased accumulation of the drug can also result (Chao, 1996; Yang *et al.*, 1993). However these resistant sublines rarely show cross resistance to the *Vinca* alkaloids even though they are often resistant to anthracyclines or epipodophyllotoxins. Usually the cross resistance is limited to alkylating agents and one other group of drugs.

Although studies of highly drug resistant sublines have been useful in identifying molecular mechanisms of MDR, they fall short of explaining the broad drug cross resistance beyond MDR that is associated with the failure of chemotherapy in the clinic. This is probably the result of differences in drug exposure. Most drug resistant sublines are developed by exposing cells continuously to high drug concentrations, forcing the cells to grow in the presence of the drug. Such treatment tends to favour the development of mechanisms such as P-glycoprotein or MRP that eliminate the drug. The drug resistance that develops under these conditions is often dependent on continued treatment for its maintenance. There are even examples of the growth of such sublines becoming dependent on the presence of drug (Roy and Horwitz, 1985). These sublines also tend to quickly lose their drug resistance when grown in the absence of drug and there is probably no clinical parallel of this. In contrast to the continuous drug exposure used in the majority of cellular models, drug exposure in the clinical setting is not continuous but is pulsed giving cells time to recover from treatment. Also the level of drug exposure in the clinical setting is generally much lower than that used to develop drug resistant cell sublines. The level of drug resistance associated with the failure of chemotherapy in the clinic is probably low in the order of 2–10 fold while the level of drug resistance in MDR sublines is often two orders of magnitude higher. A comparison of the properties generally associated with cellular and clinical MDR is given in Table I. The lack

of a cellular model with clinically relevant broad cross resistance prompted us to develop such a model taking into account the conditions listed in Table I.

Development of Extended-MDR in Haematopoietic Cells

The response to intermittent treatment with low levels of drug was initially studied in haematopoietic cell lines. The HL60 human promyelocytic leukaemia cell line was treated 6 times for 3 days with 2 ng mL⁻¹ (3.5 nM) epirubicin. This had little effect on cell growth and produced the HL60/E2 subline which was 4-fold resistant to epirubicin (Marks *et al.*, 1996). The HL60 cells were also treated for 18 hr with 40 ng mL⁻¹ (69 nM) epirubicin which is below the IC₅₀ (80 nM) for continuous exposure and also caused little toxicity. However this treatment did cause cell cycle arrest in the G₂/M phase for approximately 2 days before normal growth resumed. This was followed by 5 subsequent 18 hr treatments of 8 ng mL⁻¹ epirubicin over a period of 3 months to produce the HL60/E8 subline which was 32-fold resistant to epirubicin (Su *et al.*, 1994). A third subline was similarly developed following treatment with 8 ng mL⁻¹ (9 nM) vinblastine for 18 hr which was repeated 8 times over 3 months to give the HL60/V8 subline which was 8-fold resistant to epirubicin (Su *et al.*, 1994). As with the development of the HL60/E8 subline there was little toxicity but there was cell cycle arrest for approximately 2 days. The HL60/E2, HL60/E8 and HL60/V8 sublines have the same growth rate as the HL60 cells and they have remained stable for 3 yr without further drug treatment.

Figure 1 summarises the drug cross resistance of these sublines and shows that all 3 sublines are not only resistant to drugs normally associated with MDR but they are also cross resistant to chlorambucil, cisplatin and methotrexate. The level of resistance in most cases was modest and in the 2–30-fold range. Although this is substantially lower than that usually reported for MDR sublines, it is in the range considered to be clinically relevant. Since this was the first example of cell sublines with such a broad drug cross resistance pattern being generated by treatment with low levels of natural product drugs, it was called extended-MDR (Su *et al.*, 1994).

As well as having a broad drug cross resistance, the HL60/E8 and HL60/V8 sublines were 2- to 3-fold more resistant to γ -radiation and they had lost

Table 1. Comparison of general properties of cellular and clinical MDR

Cellular models of MDR	Clinical MDR
Continuous drug exposure	Pulsed drug exposure
Treatment with single drug	Treatment with drug combinations
Exposure to high levels of drug	Exposure to low levels of drug
High levels of drug resistance	Low levels of drug resistance
Drug resistance unstable	Drug resistance stable
Cross resistance is limited	Cross resistance is extensive

Figure 1. Drug cross resistance of the HL60 extended-MDR sublines. The sensitivity of the HL60 cells and sublines to a variety of drugs was determined using the MTT viability assay (Marks *et al.*, 1992) and the fold-resistance relative to the HL60 cells was calculated.

their ability to terminally differentiate in response to retinoic acid treatment (Su *et al.*, 1994). P-glycoprotein was expressed as determined by flow cytometry using the MRK-16 monoclonal antibody and by Western blot and immunocytochemistry with C219 monoclonal antibody (Su *et al.*, 1994) and there was also increased *MRP*-mRNA expression as determined by Northern blot analysis (Davey *et al.*, 1997). Treatment of the sublines with 50 μ M buthionine sulfoximine (BSO), which depletes cellular glutathione levels, did not cause a significant reversal of drug resistance over the 2- to 3-fold reversal in the HL60 cells except for epirubicin resistance in the HL60/E8 subline which was reversed 6-fold. This suggests that altered glutathione metabolism is probably not a major part of extended-MDR in these cells. The level of topoisomerase II- α was not decreased in the HL60/E8 and HL60/V8 sublines relative to the HL60 cells suggesting that altered topoisomerase II was probably not involved in the drug resistance of these sublines.

It is interesting that extended-MDR developed in response to treatment with low levels of drugs. In the case of the HL60/E2 subline the level of 2 ng mL⁻¹ epirubicin was well below the normal serum maintenance level of 20–30 ng mL⁻¹ achieved during treatment (Tjuljandin *et al.*, 1990). Even the higher dose of 40 ng mL⁻¹ epirubicin or 8 ng mL⁻¹ vinblastine for 18 hr had little if any toxicity suggesting that extended-MDR was induced rather than selected by these drug treatments.

To determine whether or not the ability to express extended-MDR was restricted to the HL60 cells and other cells of the promyelocytes/myeloid differentiation within haematopoiesis, a survey of cell lines representing the major haematopoietic lineages was undertaken and the potential to express the extended-MDR phenotype in response to treatment with low levels of epirubicin and vinblastine was determined. The results are summarised in Figure 2. Treatment of the U937 monocytic cell line with low levels of epirubicin and vinblastine produced the U937/E15 and U937/V8 sublines. Like the HL60/E8 and HL60/V8 sublines, both U937 sublines were stable and were maintained without further drug treatment, and they had become resistant to MDR drugs as well as to chlorambucil, cisplatin and methotrexate (Figure 3) confirming the U937 cell's capacity to express the extended-MDR phenotype (Marks *et al.*, 1996). As for the HL60 sublines, resistance was associated with increased expression of P-glycoprotein as measured

by flow cytometry using MRK-16 monoclonal and by Western blot using C219 monoclonal. However this P-glycoprotein was probably inactive since there was only a slight decrease in the accumulation of rhodamine 123 and accumulation was not increased by verapamil treatment (Marks *et al.*, 1996). *MRP* expression was also increased 3- to 4-fold in the U937 resistant sublines.

Similar treatment of the KG1a undifferentiated promyeloblastic cell line and the HEL erythromyeloblastic cell line had no effect as both lines already expressed relatively high levels of P-glycoprotein (Marks *et al.*, 1996). The K562 pluripotent stem cell line responded to intermittent treatment with low levels of epirubicin and vinblastine by expressing P-glycoprotein and becoming resistant to MDR drugs only (Marks *et al.*, 1993). A similar response was obtained in the vinblastine treated CCRF/CEM lymphocytic T-cell line with P-glycoprotein expression causing MDR while epirubicin treatment caused increased expression of *MRP* and MDR (Davey *et al.*, 1995).

Only the HL60 cells and the U937 cells, which belong to the myeloid subset, expressed extended-MDR suggesting its expression may be associated with myeloid differentiation. All the drug resistant myeloid sublines showed decreased expression of the myeloid markers CD13 and CD14 relative to the parental cells (Su *et al.*, 1994, Marks *et al.*, 1996) and this was accompanied by the appearance of glycophorin, a marker of erythroid differentiation. While this suggests that expression of extended-MDR is associated with dedifferentiation of cells, there was no expression of the stem cell marker, CD34 which has been shown to correlate with P-glycoprotein expression and poor response to therapy (List *et al.*, 1991).

Extended-MDR in Small Cell Lung Cancer Cell Lines

To extend the studies in haematopoietic cells to models of solid tumours, the small cell lung cancer (SCLC) cell lines were initially chosen since SCLC is typical of those cancers that despite showing a good initial response to induction chemotherapy, broad drug cross resistance commonly develops. This progression to broad drug cross resistance is usually rapid and suggests that SCLC cells may also develop extended-MDR.

Figure 2. Drug resistance mechanisms associated with haematopoiesis (P-gp, P-glycoprotein; +, induction).

SCLC can be divided into the 'classic' type which has a better response and prognosis, and the 'variant' type (Mattern and Volm, 1995). To investigate the involvement of extended-MDR in SCLC, the 'classic' H69 SCLC cell line (Gazdar *et al.*, 1980) and the 'variant' H82 cell line (Little *et al.*, 1983) were treated for 18 hr with 40 ng mL⁻¹ epirubicin. This caused little cytotoxicity but inhibited cell growth for approximately 1 week for the H82 cells and for 3 weeks for the H69 cells. These treated H69 cells were then treated a further 8 times for 18 hr with 8 ng mL⁻¹ epirubicin over 3 months to give the H69/E8 subline which was 2.3-fold resistant to epirubicin. In contrast, the treated H82 cells developed stable resistance to epirubicin (3.5-fold) with a further single 18 hr treatment

of 8 ng mL⁻¹ epirubicin. This produced the H82/E8 subline which did not become significantly more resistant with additional drug treatments. Both sublines have extended-MDR as they are not only resistant to MDR drugs but they are also cross resistant to chlorambucil and cisplatin (Table II). The stability of these sublines has been maintained for over 2 yr without any further drug treatment. It is interesting that extended-MDR developed faster in the 'variant' H82 cells than in the 'classic' H69 cells as this is consistent with the clinical experience of the 'classic' type of SCLC having a better response and prognosis.

P-glycoprotein was not detected in any of the SCLC cell lines or sublines. There were similar levels of *MRP*-mRNA expression in the H69 and H82

Figure 3. Drug cross resistance of the U937 extended-MDR sublines. The sensitivity of the U937 cells and sublines to a variety of drugs was determined using the MTT viability assay (Marks *et al.*, 1992) and the fold-resistance relative to the U937 cells was calculated.

cell lines. However the H82/E8 subline, but not the H69/E8 subline, showed increased *MRP*-mRNA expression. Since extended-MDR was easily induced in the 'variant' H82 cell line and this was associated with increased MRP expression, the effect of epirubicin, chlorambucil and cisplatin treatment on MRP and extended-MDR was determined in the H82 cells and the H82/E8 subline. Treatment for 1 hr with non-cytotoxic doses of either epirubicin, chlorambucil or cisplatin caused an increase in *MRP*-mRNA expression in the H82 cells as early as 4 hr after treatment. This was increased to 2-fold by 24 hr and this level of expression was maintained for at least 72 hr (Su *et al.*, 1998). Table III shows that in the case of epirubicin treatment, this increased MRP expression was accompanied by increased resistance to epirubicin (2.5-fold), chlorambucil (2.8-fold) and cisplatin (5.1-fold). Similar effects were also found with chlorambucil and cisplatin treatment. In contrast, treatment of

the H82/E8 subline did not alter its MRP expression or its resistance to these drugs (Su *et al.*, 1998).

These results suggest that both MRP and extended-MDR are part of an immediate cellular response to low, non-cytotoxic levels of epirubicin and possibly other drugs, in the H82 SCLC cells. Since MRP is not normally associated with resistance to chlorambucil or cisplatin, it also suggests that MRP along with some other drug resistance mechanism/s constitute extended-MDR. That MRP and extended-MDR can be elicited by a 1 hr treatment with such a low dose of drug is surprising. In addition, it seems this response is rapid as indicated by the increased *MRP*-mRNA. Whether this quick response is typical of most cell types or whether it is limited to cells with the potential to express the extended-MDR phenotype, remains to be determined. Irrespective of the extent of this response, it may explain the poor prognosis associated with the 'variant' form of SCLC.

Table 2. Drug cross resistance of the SCLC H69/E8 and H82/E8 sublines. Cells were incubated in drug containing medium for 5 days and their viability was determined by the MTT assay (Marks *et al.*, 1992). The mean fold-resistance and standard deviation are shown, ns = not significantly different from parental cell line

Drug	Fold-resistance (relative to parental cell line)	
	H69/E8	H82/E8
Epirubicin	2.5±0.5	3.0±0.5
Doxorubicin	2.7±0.6	3.8±0.5
Idarubicin	1.0±0.2 ns	3.2±0.6
Vinblastine	2.2±0.1	2.5±0.1
Etoposide	8.0±1.5	2.0±0.0
Paclitaxel	1.0±0.2 ns	1.6±0.2
Chlorambucil	5.6±1.6	9.8±2.5
Cisplatin	8.5±1.7	2.0±0.3

Table 3. Effect of a 1 hr pretreatment with various drugs on the drug resistance of the H82 cells. Following treatment for 1 hr with the drugs indicated, cells were incubated in drug-free medium for 24 hr before their sensitivity to a 5 day drug exposure was determined using an MTT cell viability assay (Marks *et al.*, 1992)

Pre-treatment for 1 hr with:	Fold-resistance (relative to untreated H82)		
	Epirubicin	Chlorambucil	Cisplatin
Epirubicin (69 nM)	2.5±0.04	2.8±0.30	5.1±0.3
Chlorambucil (2.5 µM)	2.6±0.10	2.8±0.30	1.6±0.2
Cisplatin (1.3 µM)	1.5±0.08	1.9±0.02	1.4±0.1

Other Cell Types with Possible Extended-MDR

The question remains—how widespread is extended-MDR? Reports from other groups of drug resistant sublines which fit the extended-MDR phenotype are summarised in Table IV. All these sublines are cross resistant to several natural product drugs and alkylating agents. Some (the MLN^R, PXN94tetR and CP2.0 sublines) show relatively low levels of drug resistance while the majority are highly resistant to many drugs. It is interesting that the sublines which most closely fit the extended-MDR phenotype, the PXN94tetR, OAW-DOX and the MCF7/ MLN^R were all the result of repeated drug treatment rather than continuous drug exposure. A variety of drugs were used to produce the resistant sublines including platinum containing drugs,

doxorubicin, melphalan and vinblastine and several drug resistance mechanisms are reported associated with these sublines including P-glycoprotein, MRP, altered glutathione metabolism, decreased topoisomerase II and decreased drug accumulation. However there is no consistent pattern of resistance mechanisms reported suggesting that there is no single resistance mechanism causing this broad drug resistance.

Factors that Promote Extended-MDR

Extended-MDR was originally used to describe cell sublines whose drug cross resistance went beyond the typical P-glycoprotein-mediated MDR and included resistance to alkylating agents such as chlorambucil, the platinum-containing drugs and antimetabolites such as methotrexate (Su *et al.*, 1994). For the time being this definition is probably a useful one as it is based on functional resistance which is clinically relevant. The term 'extended multidrug resistance' has also been used to describe drug resistance in a murine Friend erythroleukaemia cell subline that had high P-glycoprotein expression and altered topoisomerase II (Bielack *et al.*, 1995). However it is not clear whether these cells have a cross resistance phenotype similar to extended-MDR.

All the extended-MDR sublines were developed in a similar way. Rather than continuous drug exposure as is commonly the case, pulsed drug exposure was used. For two of the HL60 sublines and the SCLC sublines, the exposure time was 18 hr while for the U937 cells and the HL60/E2 subline, drug exposure was for 3 days. Further, the fast induction of extended-MDR in the H82 cells following a 1 hr drug treatment would suggest that the length of the drug pulse may not be so important in promoting the development of extended-MDR. What is probably more important is that the drug exposure is pulsed rather than continuous. A possible reason for this could be that although drug is required to induce such a response, its continued presence may inhibit essential cellular processes involved in developing extended-MDR.

Another common feature of the treatment is that relatively low doses of drugs were used. In all cases doses below the IC₅₀ for continuous drug exposure were involved in the induction of extended-MDR. This combined with a pulsed rather than a continuous treatment, means there was little cell death. Under these conditions, induction must be more important than selection in the development of extended-MDR. Even

Table 4. Examples of sublines with extended-MDR

Parent cell line	Selecting drug	Resistant subline	Drug cross-resistance ^a	Resistance mechanisms ^b	Ref.
Breast MCF7	Repeated melphalan	MLN ^R	3-melphalan, 8-mustargen, 2-cisplatin, 2.5-doxorubicin, 2.5-etoposide	No-GSH	Batist <i>et al.</i> (1989)
Ovarian PXN94	Repeated 3 day tetraplatin	PXN94tetR	24-tetraplatin, 12-bleomycin, 8-carboplatin, 8-mitomycin C, 6-cisplatin, 5-doxorubicin, 4-5-fluorouracil, 2-etoposide, 2-vinblastine	Decreased accumulation, No-GSH, No-P-gp	Mellish <i>et al.</i> (1994)
Ovarian OAW-42	Repeated doxorubicin	OAW-DOX	93-doxorubicin, 616-paclitaxel, 12-cisplatin, 34-colchicine, 118-vincristine	P-gp, GSH, Topo II, Thymidylate synthase	Masanek <i>et al.</i> (1997)
Ovarian A2780	Continuous cisplatin	CP20, CP70, C30 to C200*	* 420-cisplatin, 500-melphalan, 78-doxorubicin, 446-mitoxantrone, 852-etoposide, 38-paclitaxel	GSH, No-P-gp, No-MRP	Hamaguchi <i>et al.</i> (1993)
Ovarian SKOV3	Continuous cisplatin	SKOV3/CIS	3.7-cisplatin, 1.8-chlorambucil, >1000-doxorubicin, >1000-vinblastine, >100-paclitaxel	P-gp	Yang and Page (1995)
B-Lymphoma HOB1	Continuous doxorubicin	HOB1/ADR	240-doxorubicin, 133-colchicine, 500-vinblastine, 200-vincristine, 65-cisplatin, 25-mitomycin	P-gp, No-decreased doxorubicin accumulation	Chao (1995)
B-Lymphoma HOB1	Continuous vincristine	HOB1/VCR	208-vincristine, 214-puromycin, 125-colchicine, 75-doxorubicin, 67-cisplatin, 40-mitomycin	Decreased cisplatin accumulation, P-gp, GSH	Chao (1996)
Colon LoVo	Continuous cisplatin	CP2.0	6-cisplatin, 2.3-mustargen, 2.6-5-fluorouracil, 2.6-doxorubicin, 7.7-vincristine, No-etoposide	Decreased cisplatin accumulation, P-gp, GSH	Yang <i>et al.</i> (1993)

^a Number preceding drug is fold-resistance, No- = no resistance.

^b GSH = altered glutathione metabolism, P-gp = P-glycoprotein, MRP = multidrug resistance-associated protein. Topo II = decreased topoisomerase II levels, No- = no change.

though the first drug treatment caused cell cycle arrest during the development of the HL60/E8, HL60/V8, H69/E8 and H82/E8 sublines, cell cycle arrest is not necessary for the development of extended-MDR since the HL60/E2 subline developed without cell cycle arrest.

The drugs so far implicated in the development of extended-MDR are epirubicin and vinblastine. However the possibility that other drugs may also induce extended-MDR has not yet been tested. The fact that a 1 hr treatment with chlorambucil caused an increase in both MRP expression and epirubicin and chlorambucil resistance suggests that chlorambucil may also be able to induce extended-MDR. In addition, the

sublines listed in Table IV were generated by treatment with cisplatin, tetraplatin, doxorubicin, melphalan and vincristine. Since these drugs have different intracellular targets and mechanisms of action, the type of drug may not be important for the development of extended-MDR. However until other types of drugs are assessed for their ability to induce extended-MDR, little can be concluded on what drug properties promote extended-MDR.

Studies so far have shown that the expression of the extended-MDR phenotype in haematopoietic cells is limited to the myeloid lineage. For cell lines established from solid tumours little is known about the extent of this phenotype. Apart from SCLC having

the ability to express extended-MDR, preliminary evidence suggests that MCF7 breast cancer cells do not respond to pulsed low level drug treatment to produce the extended-MDR phenotype (unpublished observations). However, the MCF7/MLN^R listed in Table IV does have a drug cross resistance profile consistent with extended-MDR. The majority of cell lines listed in Table IV are of ovarian origin suggesting this cell type may have the potential to express extended-MDR.

Molecular Mechanisms of Extended-MDR

All sublines expressing extended-MDR have elevated levels of *MRP*-mRNA. Further, the upregulation of MRP and resistance to MDR and non-MDR drugs in the SCLC H82 cells in response to a 1 hr treatment with epirubicin or chlorambucil would suggest MRP is involved in extended-MDR. Since extended-MDR involves resistance to drugs not normally associated with MRP such as chlorambucil and cisplatin, there must be other resistance mechanisms operating in addition to MRP. Alternatively, the possibility that MRP causes resistance to a broader range of drugs, including chlorambucil and cisplatin, has been previously suggested (Shen *et al.*, 1996). This is based on a possible interaction between MRP and glutathione since glutathione is associated with resistance to a broader range of drugs including cisplatin and chlorambucil. This concept is supported by the coordinate upregulation of γ -glutamylcysteine synthetase, a key enzyme in glutathione synthesis, and MRP in the HL-60/R-CP subline which is resistant to cisplatin (Ishikawa *et al.*, 1996). Further, the ATP-dependent transport of glutathione-chlorambucil conjugate has recently been demonstrated in isolated membrane vesicles from MRP-transfected HeLa cells (Barnouin *et al.*, 1997). Therefore MRP may mediate extended-MDR through its association with glutathione. Against this circumstantial evidence is the fact that the vast majority of MRP-overexpressing cell sublines are not resistant to chlorambucil or cisplatin.

One other explanation could be that an MRP-like protein may cause resistance to cisplatin and chlorambucil. Evidence for this is that the multiorganic anion transporter (cMOAT), also known as cMRP or MRP₂ (Keppler and König, 1997) which is related to MRP, has been associated with resistance to cisplatin (Taniguchi *et al.*, 1996). This raises the possibility that cMOAT, or one or more of the recently identified rel-

atives of MRP (Kool *et al.*, 1997), may be involved in extended-MDR.

Altered glutathione metabolism could in combination with MRP, explain extended-MDR since glutathione is known to protect cells against drugs such as chlorambucil and cisplatin. However altered glutathione metabolism seems not to be a consistent feature of extended-MDR cells. Depletion of cellular glutathione with BSO treatment had little effect on the drug resistance levels of the HL60 and U937 sublines while it did cause reversal of epirubicin and chlorambucil resistance in the SCLC H82/E8 and H69/E8 sublines. The lack of a consistent association between altered glutathione and drug resistance is also shown for the sublines in Table IV.

Other resistance mechanisms involving increased repair of damaged DNA could also be part of extended-MDR. Again there are no consistent associations for these mechanisms either for the cell sublines listed in Table IV or for the sublines described in detail. Rather than one other mechanism being associated with MRP to cause extended-MDR, there may be several other mechanisms involved.

While the underlying mechanisms of extended-MDR are not yet characterised, a possibility which cannot be overlooked is that the phenotype may be mediated by changes in oncogene expression and their effect on the cell's ability to undergo apoptosis. Inhibition of apoptosis is commonly reported to cause increased drug resistance. Also the expression of several drug resistance mechanisms has been reported to be associated with changes in the expression of oncogenes involved in apoptosis (Kellen, 1994). Drugs that are generally thought to cause cell death mainly by apoptosis include etoposide, vincristine, doxorubicin, cisplatin and chlorambucil (Hannun, 1997), drugs associated with extended-MDR.

The term 'extended-MDR' refers to drug treated cells that show cross-resistance to a broader range of drugs than simply the natural product drugs and we have demonstrated induction of this phenotype is associated with some specific cell types. However this response may be more general as many highly drug resistant sublines show an underlying low level resistance to non-MDR drugs. It may be that extended-MDR is the initial response of all cells to intermittent treatment with most chemotherapeutic drugs. That MRP may be involved in such a response is consistent with its ubiquitous expression. This proposal is supported by the clinical failure of chemotherapy to cure cancer. Only by understanding the mechanism by

which cells are able to avoid this initial insult, can we provide alternate, more successful, treatments.

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