Drug Resistance and its Clinical Circumvention

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Systemic therapy with cytotoxic drugs is the basis for most effective treatments of disseminated cancers. Additionally, adjuvant chemotherapy can offer a significant survival advantage to selected patients, following the treatment of localized disease with surgery or radiotherapy, presumably by eliminating undetected minimal or microscopic residual tumor. However, the responses of tumors to chemotherapeutic regimens vary, and failures are frequent owing to the emergence of drug resistance.

Patterns of treatment response and tumor sensitivity are conveniently divided into three groups. First, with modern treatments, prompt cyto reduction and cures are common for some intrinsically drug-sensitive tumors, such as childhood acute lymphoblastic leukemia (ALL), Hodgkin’s disease, some non-Hodgkin’s lymphomas, and testicular cancer. A second group comprises tumors such as breast carcinomas, small-cell lung cancers, and ovarian carcinomas, which are also usually highly responsive to initial treatments but more often become refractory to further therapy. Relapses in either group of tumors, particularly during or shortly after the completion of therapy, generally herald the emergence of tumor cells that are resistant to the antineoplastic agents used initially and often to drugs to which the patient was never exposed. Therefore, success with conventional salvage chemotherapies has been limited. Finally, a third common pattern of drug sensitivity is found in tumors that are intrinsically resistant to most chemotherapeutic agents. This group is represented by malignancies such as non–small-cell lung cancers, malignant melanoma, and colon cancer. For these tumors, the number of active antineoplastic agents is low, and significant chemotherapeutic responses are effected only in a minority of cases.

The phenomenon of clinical drug resistance has prompted studies to clarify mechanisms of drug action and to identify mechanisms of antineoplastic resistance. It is expected that through such information, drug resistance may be circumvented by rational design of new non–cross-resistant agents, by novel delivery or combinations of known drugs and by the development of other treatments that might augment the activity of or reverse resistance to known antineoplastics. Multiple mechanisms of antineoplastic failure have been identified using in vitro (tissue culture) and in vivo (animal and xenograft) models of antineoplastic resistance. Table 48-1 categorizes these general mechanisms of drug resistance. Considered here are mechanisms involving anatomic, pharmacologic, and host–tumor interactions that are uniquely pertinent to patients and to in vivo models of drug resistance, as well as cellular mechanisms that can be described at the molecular level. These mechanisms are frequently interrelated as, for example, altered gene expression must ultimately underlie most of the cellular and biochemical mechanisms listed in Table 48-1. Furthermore, multiple independent mechanisms of antineoplastic resistance may coexist in a population of tumor cells.

While mechanisms of drug resistance have been largely determined in experimental systems, many have been implicated in at least some examples of clinical chemotherapeutic failure. Evidence that bears upon these mechanisms of resistance as well as strategies to circumvent them are discussed below. First, we discuss the general mechanisms of cellular drug resistance and then some specific examples in the sections that follow. Additionally, the important concept of resistance to multiple antineoplastic agents, resistance to specific classes of drugs, and resistance mechanisms unique to in vivo situations are discussed.

GENERAL MECHANISMS OF DRUG RESISTANCE

Experimental selection of drug resistance by repeated exposure to single antineoplastic agents will generally result in cross-resistance to some related agents of the same drug class. This phenomenon is explained on the basis of shared drug transport carriers, drug metabolizing pathways, and intracellular cytotoxic targets of these structurally and biochemically similar compounds. Generally, the resistant cells retain sensitivity to drugs of different classes with alternative mechanisms of cytotoxic action. Thus, cells selected for resistance to alkylating agents or antifolates will usually remain sensitive to unrelated drugs, such as anthracyclines. Exceptions include emergence of cross-resistance to multiple, apparently structurally and functionally unrelated drugs, to which the patient or cancer cells were never exposed during the initial drug treatment. Despite apparent differences in the families of drugs associated with multidrug resistance (MDR) phenotypes, when the mechanisms underlying these phenotypes are identified, we frequently discover that the involved antineoplastic agents share common metabolic pathways, efflux transport systems, or sites of cytotoxic action. Conceptually then, the targets of MDR mechanisms are similar to the targets of single-agent resistance mechanisms.

In this section, we describe broadly defined processes related to drug resistance and a few specific examples. A more comprehensive discussion follows in the sections on resistance to specific classes of drugs.

DECREASED DRUG ACCUMULATION

Decreased intracellular levels of cytotoxic agents is one of the most common mechanisms of drug resistance. Polar, water-soluble drugs cannot penetrate the lipid bilayer of the cell membrane and

<table>
<thead>
<tr>
<th>Table 48-1 General Mechanisms of Drug Resistance</th>
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<tr>
<td><strong>Cellular and biochemical mechanisms</strong></td>
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<tr>
<td>Decreased drug accumulation</td>
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<tr>
<td>Decreased drug influx</td>
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<tr>
<td>Increased drug efflux</td>
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<td>Increased intracellular trafficking of drug</td>
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<td>Decreased drug activation</td>
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<td>Increased inactivation of drug or toxic intermediate</td>
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<td>Increased repair of or tolerance to drug induced damage to</td>
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<td>Deoxyribonucleic acid (DNA)</td>
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<td>Protein</td>
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<td>Membranes</td>
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<td>Drug targets altered (quantitatively or qualitatively)</td>
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<tr>
<td>Altered cofactor or metabolite levels</td>
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<tr>
<td>Altered downstream effectors of cytotoxicity</td>
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<td>Altered signalling pathway and/or apoptotic responses to drug insult</td>
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<tr>
<td>Altered gene expression</td>
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<tr>
<td>DNA mutation, amplification, or deletion</td>
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<tr>
<td>Altered transcription, postranscription</td>
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<tr>
<td>processing or translation</td>
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<tr>
<td>Altered stability of macromolecules</td>
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<tr>
<td>Mechanisms relevant in vivo</td>
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<tr>
<td>Pharmacologic and anatomic drug barriers (tumor sanctuaries)</td>
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<td>Host–drug interactions</td>
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<td>Increased drug inactivation by normal tissues</td>
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<tr>
<td>Decreased drug activation by normal tissues</td>
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<tr>
<td>Relative increase in normal tissue drug sensitivity (toxicity)</td>
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<td>Host–tumor interactions</td>
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require specific mechanisms of cell entry; resistance to these drugs, therefore, can be mediated by the downregulation of these uptake mechanisms. For example, decreased influx via a high-affinity folate-transport system, as well as via a reduced folate carrier, is a well-described cause of methotrexate resistance. A deficient membrane transport system has similarly been identified in cells resistant to nitrogen mustard. For hydrophobic, nonpolar drugs that can easily diffuse across the cell membrane, intracellular drug concentration can be reduced by increasing the activities of drug efflux pumps. For example, overexpression of the P-glycoprotein (MDR1) drug efflux pump (classic MDR) is an important example of this mechanism of resistance.

**Altered Drug Metabolism** Modified drug activation, drug inactivation, or cofactors can confer resistance to selected antineoplastic agents. For example, many antimitabolites and some alkylating agents (eg, cyclophosphamide) are administered as prodrugs, which must be activated to their cytotoxic forms by the targeted tumor or by other tissues. Resistance to some nucleobase drugs is associated with decreased conversion of these analogs to their cytotoxic nucleoside and nucleotide derivatives by kinases and phosphoribosyl transferase salvage enzymes. Similarly, cellular sensitivity to the cytotoxicity of the topoisomerase I poison CPT-11 is in part governed by the level of carboxyesterase—an activity necessary to convert CPT-11 to its active metabolite, SN-38. Furthermore, enhanced inactivation of pyrimidine and purine analogs by elevated deaminases is linked to resistance toward these agents. Finally, cofactor levels may modify drug toxicity. For example, optimal formation of inhibitory complexes between 5-fluorodeoxyuridine monophosphate (FdUMP) and its target enzyme, thymidylate synthase, require the cofactor 5,10-methylene tetrahydrofolate.

**Increased Repair or Cellular Tolerance or Drug-Induced Damage** Cells contain multiple complex systems involved in the repair of membrane and deoxyribonucleic acid (DNA) damage. Because such damage may occur as a direct or secondary consequence of cytotoxic drug action, altered intrinsic repair mechanisms can influence drug sensitivity. For example, resistance to cisplatin, a drug whose cytotoxic action is thought to involve intrastrand DNA cross-linkages (see below), is associated with altered activities presumed to reflect increased DNA repair. Conversely, defects in mismatch repair are associated with tolerance to cisplatin-induced DNA damage. It is hypothesized that in this form of platinum resistance, the repair system is unable to recognize platinum-DNA adducts, and that this defect leads to the failure to activate the normal, programmed cell death (apoptotic) response.

Different classes of anticancer drugs initiate their cytotoxicities through a variety of primary molecular targets. Increasingly, however, the view is held that most, if not all, cancer drugs ultimately effect cell death via common downstream signaling pathways associated with programmed cell death or apoptosis. Drug insults may lead to several alternative cellular responses, including cell-cycle arrest and activation of repair processes, or active cell suicide by apoptosis. Mutations or altered expression levels of the key genes regulating these alternative responses to drug-induced stress—genes that include p53, p21^cip/WAF^, and bcl2 family genes—can profoundly influence cellular sensitivity or resistance to cancer drugs.

**Altered Drug Targets** The mechanisms of cell kill of several antineoplastic drugs involve interactions between the drug and an essential intracellular enzyme. These interactions result in alteration or inhibition of normal functions. Quantitative or qualitative changes in these enzyme targets of antineoplastic drugs can compromise drug efficacy. These changes have been demonstrated in several enzymes associated with drug-resistant cells, including dihydrofolate reductase, thymidylate synthase, and topoisomerase I and II. Perhaps surprisingly, alteration of the drug target has become recognized as a mechanism of resistance to newer molecularly targeted chemotherapy. Clinical resistance to the BCR-ABL kinase inhibitor Gleevec (imatinib mesylate) results from the development of mutations in the kinase’s drug-binding site. Thus, the newer targeted therapy approaches can be just as vulnerable to the development of drug resistance as older, standard cytotoxic drugs.

**Altered Gene Expression** The cellular mechanisms of drug resistance outlined above depend upon altered levels or function of key gene products. These alterations may result from changes that occur at any point along the pathways of gene expression and regulation. Indeed, multiple molecular processes are involved in examples of drug resistance, including DNA mutation, deletion, or amplification; altered transcriptional or posttranscriptional control of ribonucleic acid (RNA) levels; and altered posttranslational modifications of proteins. The prevalence of these changes reflects the phenotypic and genetic instability of cancer cells under the selective, and perhaps mutagenic, pressures of xenobiotic toxin and drug exposure.

**RESISTANCE TO MULTIPLE DRUGS**

De novo and acquired cross-resistance to multiple antineoplastic agents can result from several alternative factors and processes. Accordingly, we have grouped the major patterns of cross-resistance into several categories, on the basis of their presumed underlying mechanisms (Table 48-2). First, MDR patterns of cross-resistance are frequently associated with decreased drug accumulation, usually because of increased drug efflux. Classic MDR associated with resistance to drugs listed in Table 48-3 is mediated by P-glycoprotein (MDR1, P-170). More recently, a similar but distinct MDR phenotype was attributed to the energy-dependent drug efflux activities of multidrug resistance protein (MRP) family members. Another overlapping but discrete resistance MDR phenotype is associated with increased expression of the recently isolated putative efflux transporter, breast cancer resistance protein (BCRP). MDR has also been described in association with overexpression of the lung resistance protein (LRP). The mechanism of LRP-associated resistance is unclear, and whether LRP alone is sufficient to confer resistance is unknown. It is speculated that as a major vault protein, LRP is involved in nucleocytoplasmic transport and may be able to prevent entry of drugs into the nucleus.

### Table 48-2 Mechanisms of Multidrug Resistance (MDR)

<table>
<thead>
<tr>
<th>Resistance associated with decreased drug accumulation</th>
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<tr>
<td>- ABC transporter-mediated resistance</td>
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<tr>
<td>- P-glycoprotein/MDR1-mediated classic MDR</td>
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<tr>
<td>- MRP family member-mediated MDR</td>
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<tr>
<td>(currently at least 3 members, MRP1, 2, and 3 implicated in MDR drug efflux detoxification)</td>
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<tr>
<td>- BCRP-mediated MDR (putative ABC half-transporter implicated in mitoxantrone and anthracycline resistance)</td>
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<td>MDR associated with altered topoisomerases</td>
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### Table 48-3 Cross-Resistance Pattern of Classic (P-glycoprotein–mediated) MDR

<table>
<thead>
<tr>
<th>Class</th>
<th>Drug</th>
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<tr>
<td>Anthracyclines</td>
<td>Doxorubicin</td>
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<td></td>
<td>Daunorubicin</td>
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<td></td>
<td>Mitoxantrone</td>
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<td>Antibiotics</td>
<td>Actinomycin D</td>
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<td></td>
<td>Plicamycin</td>
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<td>Antimicrotubule drugs</td>
<td>Vincristine</td>
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<td></td>
<td>Vinblastine</td>
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<td></td>
<td>Colchicine</td>
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<tr>
<td>Epipodophyllotoxins</td>
<td>Etoposide</td>
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<tr>
<td></td>
<td>Tenoposide</td>
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CLASSIC (P-GLYCOPROTEIN–DEPENDENT) MDR

An in vitro model of MDR was described by Biedler and coworkers three decades ago.52 In these studies, cultured cells selected for resistance by exposure to actinomycin D developed cross-resistance to a surprising array of structurally diverse compounds, including vinca alkaloids, puromycin, daunomycin, and mitomycin C. Subsequently, induction of this pattern of cross-resistance has been observed by numerous investigators, who have selected cells in the presence of the same and other drugs. Generally, exposure of cells to any of the drugs (many of which are listed in Table 48-3) related to this MDR phenotype can result in cross-resistance to all other members of the phenotype.8,9 Drug transport studies using parental and MDR cells demonstrate that the reduced cytotoxicity of these drugs is the result of decreased drug accumulation secondary to enhanced drug efflux.43,44 Furthermore, the emergence of MDR has been associated with increased levels of a membrane-bound glycoprotein, P-glycoprotein (P-170 or MDR1 protein).

Although it is widely accepted that P-glycoprotein mediates an energy-dependent decrease in drug accumulation, there is considerable debate on the precise mechanism(s) involved. Drugs associated with the classic MDR phenotype are generally freely permeable to the plasma membrane. In one model, drugs in the cytosol may be recognized by P-glycoprotein and exported back across the plasma membrane in association with adenosine triphosphate (ATP) hydrolysis.9 Another proposal, termed the “hydrophobic vacuum cleaner” model, suggests that the lipid-soluble drugs may be recognized by P-glycoprotein within the plasma membrane and expelled without ever entering the cytoplasm.9,45 A third model is the lipid “flippase” model, in which the role of P-glycoprotein is to merely flip drugs from the inner leaflet of the cell membrane to the outer leaflet, and therefore drive diffusion of drug out of the cell.46 Numerous other mechanisms of P-glycoprotein–dependent drug transport have been proposed, including processes in which drug efflux is indirectly influenced by P-glycoprotein–mediated changes in membrane potential or chloride channels.37–50 Finally, it has been noted that if the freely diffusible, lipid-soluble drugs are the substrates of P-glycoprotein, then huge expenditures of energy would be required to maintain reduced drug accumulation in cells continually exposed to extracellular drug. To obviate this thermodynamic obstacle, it has been suggested that the true substrates of P-glycoprotein–mediated efflux may not be the parent drugs, but rather the previously unidentified amphiphilic and membrane-impermeable drug conjugates formed within the cell.51

Regardless of the mechanistic details, a great deal of evidence supports the consensus view that P-glycoprotein is the energy-dependent drug efflux pump responsible for MDR. First, gene transfer experiments show that the expression of P-glycoprotein genes is sufficient to confer drug resistance.52,53 Second, P-glycoprotein belongs to a multigene family of transport proteins (ABC transporters), all of which share sequence homology with bacterial transport proteins.54,55 Third, photoaffinity labeling experiments demonstrate direct binding of drugs to P-glycoprotein.56 Finally, the distribution of P-glycoprotein on the luminal surfaces of normal tissues including renal tubules, colon, small intestine, and bile canaliculi is consistent with its proposed role in excretory transport.57 Thus, P-glycoprotein appears to fulfill the requirements predicted of a membrane-bound energy-dependent drug exporter. Although there are two human MDR genes, only MDR1 confers drug resistance.8,9 P-glycoprotein–associated MDR displays significant phenotypic heterogeneity. The relative degree of cross-resistance to the drugs listed in Table 48-3 varies based on the cell line and the selecting drug. While the level of drug resistance is roughly correlated with the level of P-glycoprotein expression, protein and RNA levels may be disproportionately higher or lower than expected for the level of resistance observed. This phenotypic diversity may be the result of both MDR1 mutations and of posttranslational modifications of the MDR1 gene product. Mutations in the coding region of the MDR1 gene have been reported to alter the relative resistance patterns of cells.58 P-glycoprotein can be phosphorylated by protein kinase C59,60 and by a novel membrane associated protein kinase.61 Transport studies on MDR cells treated with protein kinase C activators and inhibitors, as well as with inhibitors of protein phosphatases, show that increased phosphorylation of P-glycoprotein is associated with decreased vinblastine accumulation.62,63 Other cofactors involved in the augmentation of P-glycoprotein function have been proposed but not yet identified.51,64

A thorough understanding of the regulation of P-glycoprotein production and the means to suppress its expression might significantly influence future cancer treatment strategies. Studies addressing this issue have shown that high levels of P-glycoprotein expression in vitro are often associated with MDR gene amplification and transcriptional activation.5,9 Increased expression of P-glycoprotein can also be stimulated by heat shock.65 Heavy metals, cytotoxic drugs,56–68 regenerating liver,66,67 differentiating agents,69–71 and by repeated exposure to ionizing radiation.72 However, the responses to these treatments appear to vary between species and are cell line specific. Thus, predictable modulation of MDR gene expression is not yet possible. Under certain conditions in some cells, the MDR1 promoter activity can be regulated by altered expression of oncogenes (raf and ras) and the tumor-suppressor gene, p53.73–77

A considerable literature has accumulated concerning the importance of P-glycoprotein in human cancer. P-glycoprotein RNA or protein has been detected in tumor specimens derived from patients with acute and chronic leukemias,78–80 ovarian cancer,81 multiple myeloma,82 breast cancer,83,84 neuroblastoma,85 soft tissue sarcomas,86 renal cell carcinoma,87 and others.88 Although the numbers of patients with particular tumors in these studies were small, the results have tended to link increased P-glycoprotein expression with a history of prior therapy (usually with MDR-associated drugs) or toxin exposure, and poorer treatment outcome. In general, the relationship between increased P-glycoprotein and adverse outcome in human cancers is strongest in hematologic malignancies. Recently, three prospective studies have shown that increased P-glycoprotein in patients with acute myelogenous leukemia (AML) is associated with decreased complete remission rates and reduced remission duration with use of conventional chemotherapy.89–91 This correlation has also been demonstrated in adult multiple myeloma, lymphoma, and pediatric ALL92–94 Moreover, efforts to reverse clinical resistance to chemotherapy using P-glycoprotein inhibitors (see below) have similarly been most promising in the treatment of selected hematologic malignancies.95,96–97

Among solid tumors, the relationship between P-glycoprotein expression and response to therapy is less convincing,98 although significant correlations between P-glycoprotein and adverse outcome in pediatric rhabdomyosarcoma and neuroblastoma have been reported.92 Although P-glycoprotein was frequently present in tumor specimens from both treated and untreated patients with neuroblastoma, P-glycoprotein RNA tended to be higher in patients treated with regimens that included doxorubicin than in untreated patients.85 Moreover, in patients with advanced neuroblastoma, P-glycoprotein expression has been strongly associated with aggressive biologic behavior, poor treatment response, and poor outcome.99 The impressive correlations between P-glycoprotein expression and aggressive neuroblastoma persisted even when the data were corrected, by multivariate analyses, for other confounding prognostic features. However, the significance of MDR1 expression in neuroblastomas is controversial as other data have suggested the opposite—that increased MDR1 expression is associated with more favorable clinical variables in patients with neuroblastoma.100 In tumor specimens obtained from patients with soft-tissue sarcomas,86 the presence of P-glycoprotein was associated with anthracycline pretreatment, increased rate of remission induction failure, and increased frequency of relapse. In the more than 400 tumor specimens that were tested for P-glycoprotein RNA levels in a large study,88 increased levels of MDR1 RNA were more prevalent in tumors that tended to be intrinsically resistant to therapy (colon, renal, adrenal, hepatic, and pancreatic cancers) as compared with intrinsically sensitive tumors. Furthermore, P-glycoprotein RNA was often increased in tumors at relapse (acute leukemias, breast cancer, neuroblastoma, pheochromocytoma, and nodular poorly differentiated lymphoma).
Consequently, the available evidence indicates that P-glycoprotein overexpression is associated with clinical evidence of drug resistance and treatment failure in a significant number of patients—especially, selected groups with hematologic malignancies. However, additional and prospective studies are required to fully evaluate the clinical significance of P-glycoprotein in human cancer. These studies should include standardized P-glycoprotein RNA, protein and functional determinations in clinical specimens, and clearly defined clinical outcomes. Such studies will help establish cancers for which the determination of P-glycoprotein levels in patients at diagnosis or relapse may have an important role in the design of treatment protocols.

**Multidrug Resistance Protein Family**

Similar phenotypes of multiple resistance to antineoplastic agents have been described that are associated with the expression of other membrane proteins. In many of these examples, resistance occurs independently of P-glycoprotein expression. A distinct gene, *mrp1* (multidrug resistance protein 1 or multidrug resistance-associated protein 1), was isolated from a doxorubicin-selected MDR lung cancer cell line. Except for the absence of P-glycoprotein expression, the phenotype of this cell line, which includes the property of reduced drug accumulation, was similar to classic MDR. The *mrp1* gene encodes a 190-kilodalton (kDa) transmembrane protein, whose structure is strikingly homologous to P-glycoprotein/MDR1 and other members of the ATP-binding cassette (ABC) transmembrane transporter proteins. Primary sequence analysis predicts the transmembrane structure shown in Figure 48-1. The structure, supported by immunohistochemical data, includes 11 plus 4 (or, alternatively, 11 plus 6) transmembrane domains with 2 cytosolic ATP-binding sites. Increased MRP1 expression is associated with MDR, and decreased MRP1 expression is associated with reversion to drug sensitivity. Gene transfer experiments establish that MRP1 can confer MDR to a variety of drugs, including anthracyclines, epipodophyllotoxins, and vinca alkaloids. Transport studies indicate that MRP1 is involved in ATP-dependent efflux of some native natural product anticancer drugs. Additionally, MRP1 is an ATP-dependent transporter of a variety of anionic conjugates of drugs and other xenobiotics—conjugates that include glutathione conjugates, glucuronides, and sulfates. Thus, MRP1 is an important xenobiotic-conjugate transport pump that is involved in efflux detoxification of a wide range of cellular poisons, including anticancer drugs and their conjugates. The significance of these conjugate substrates is further discussed in a following section. In contrast to P-glycoprotein, whose substrates are generally lipophilic neutral or cationic compounds, MRP1 preferentially recognizes amphiphilic organic anions including the conjugates described above.

Vincristine are also substrates of MRP1, reduced glutathione is required for their transport. Although no covalent linkage between glutathione and vincristine is observed, it is believed that both glutathione and the neutral drug must be simultaneously present to effect efflux, and that they both may be cotransported by MRP1.

MRP1 is ubiquitously expressed in tumor and normal tissues. The importance of MRP1 overexpression in clinical drug resistance is unknown. However, because levels of MRP1 expression vary widely in tumor cells, MRP1 may be a significant mediator of drug resistance in human cancer.

There are at least five other human MRP isoforms identified. Among them, MRP2 (cMOAT) and MRP3 are also capable of supporting efflux detoxification of cancer drugs, including epipodophyllotoxins (MRP2 and 3), doxorubicin, and cisplatin (MRP2). Recent results indicate that MRP1, MRP2, MRP3 and MRP4 can all act as methotrexate efflux pumps and can confer resistance to methotrexate. Unlike MRP1, which is expressed on the basolateral plasma membrane surface of polarized cells, MRP2 is normally targeted to the apical membrane surface of bile canaliculus and renal tubular epithelium. MRP3 is localized to the basolateral surface in various tissues, including the colon, liver, and pancreas, and can also confer low-level resistance to etosine and teniposide.

**MDR Associated with Topoisomerase Poisons**

Topoisomerases are nuclear enzymes that catalyze the formation of transient single- or double-stranded DNA breaks, facilitate the passage of DNA strands through these breaks, and promote rejoining of the DNA strands. As a consequence of these activities, topoisomerases are thought to be critical for DNA replication, transcription, and recombination. The cytotoxicity of many drugs that target topoisomerases, a class of drugs here termed topoisomerase poisons (Table 48-3), is thought to depend on the DNA cleavage activities of topoisomerases. There are two classes of mammalian enzymes, topoisomerases I and II. Topoisomerase I catalyzes the formation of single-stranded DNA breaks, while topoisomerases II (α and β isoforms) catalyze both single- and double-stranded breaks. During the cleavage reactions reversible DNA-topoisomerase complexes (cleavable complexes) can be stabilized by interactions with topoisomerase poisons. The formation of these stabilized DNA-topoisomerase–drug complexes is thought to initiate the production of lethal DNA strand breaks. Of the chemotherapeutic drugs that affect topoisomerase activities, the topoisomerase II poisons have been the most important clinically. A partial list of these agents, which include DNA intercalating and nonintercalating drugs, appears in Table 48-3. A growing list of useful topoisomerase I poisons are now available, including topotecan, CPT-11 (irinotecan), and SN-38.

Several laboratories have described an MDR pattern characterized by resistance of cells to several or all of the drugs listed in Table 48-3. It is readily apparent that many of these topoisomerase II–targeting drugs are also mem-

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**Figure 48-1** Models of P-glycoprotein and MRP1.
breaks. Indeed, the normal downregulation of epipodophyllotoxins. Collectively, these data indicate that reduced topoisomerase protein levels following exposure to m-AMSA or microtubule alteration exists. The mechanism of resistance to topoisomerase II poisons is thought to involve altered topoisomerase II activity. Both qualitative and quantitative changes in enzyme activity have been demonstrated in resistant cell lines. Reduced levels of topoisomerase activity are associated with decreased drug-induced DNA strand breaks, as well as reduced drug cytotoxicity. Other studies implicate intrinsic changes in drug-induced catalytic properties or associated cofactors as the basis of drug resistance in some cells. The nature of the topoisomerase II alterations may influence the cross-resistance patterns observed. For example, cells that develop alterations in topoisomerase II following exposure to m-AMSA (amsacrine) may show cross-resistance to other intercalating topoisomerase II poisons, but not to epipodophyllotoxins. Collectively, these data indicate that reduced topoisomerase protein levels or selectively altered enzyme activities influencing drug–enzyme interactions may render cells relatively more resistant to drugs by interfering with the formation of stable cleavable complexes and hence cytotoxic DNA strand breaks. Indeed, the normal downregulation of topoisomerase II in nondividing cells may explain the relative insensitivity to topoisomerase II poisons of some solid tumors containing a large proportion of quiescent cells. Finally, there are two mammalian isozymes of topoisomerase II, a 170-kDa form (topoisomerase IIA) and a 180-kDa form (topoisomerase IIB).

These isozymes differ with respect to their regulation during the cell cycle and their relative sensitivities to topoisomerase II poisons. Hence, the relative levels of the specific topoisomerase II isozymes as well as the total topoisomerase II activity may be significant determinants of the sensitivity of tumor cells to topoisomerase II drugs.

Several reports suggest the molecular bases of drug resistance associated with qualitatively altered topoisomerase II. Point mutations leading to amino acid substitutions in topoisomerase IIA α and IIB α isoforms are associated with drug resistance in intact cells and, in some cases, with altered enzymatic activities in vitro, the exact mechanism of drug resistance and the relationship of these mutations to a specifically altered enzymatic property are incompletely understood. Moreover, the relevance for clinical drug resistance of these topoisomerase II mutations identified in experimentally drug-selected resistant cell lines is unknown. Indeed, one study of topoisomerase IIA α mutations derived from leukemic blasts of 15 relapsed patients failed to identify mutations in either of the above two regions implicated in experimental drug resistance. Other qualitative alterations in topoisomerase II activity and structure have been described in cell lines selected for resistance to topoisomerase II poisons. These alterations include a selective decrease in nuclear matrix–associated topoisomerase IIα1 and a truncated form of topoisomerase IIα. In some resistant cell lines, cytoplasmic or membrane components may be responsible for the altered topoisomerase II α activity implicated in the emergence of drug resistance. Alternatively, altered subcellular localization of topoisomerase II isoforms or posttranslational phosphorylation have been reported in association with some etoposide-resistant cell lines.

The cytotoxicity of topoisomerase II poisons is believed to depend on the formation of DNA strand breaks secondary to stabilization of the reversible enzyme-DNA cleavable complex. It is thought that a collision between the complex and the DNA replication fork is necessary to generate the cytotoxic lesions. If DNA replication is delayed or altered until after the drug is cleared, the cleavable complex can be reversed and the cytotoxic lesion does not form. Thus, altered DNA replication or repair timing could also mediate topoisomerase II poison resistance.

A new family of drugs targeting topoisomerase II function has emerged that includes fosfotitromycin, merbarone, aclacinomycin, and hib (2,6-dioxopiperazine) derivatives (eg, ICRF 193 and ICRF 187). In contrast to the topoisomerase II poisons that stabilize cleavable complexes (see above and Table 48-4), this new family of drugs target the catalytic cycle of topoisomerase II activity in which DNA strands are intact. Because the toxicity of these “catalytic inhibitors” is independent of cleavable complex stabilization, cross-resistance with the topoisomerase II poisons is less likely.

The cytotoxic agent camptothecin enhances topoisomerase I–mediated strand breaks. Earlier, host toxicity prohibited the clinical use of such topoisomerase I poisons. However, the prospect of less-toxic analogs of this drug that maintain a high level of activity against topoisomerase I–rich human cancer cells has renewed interest in the clinical application of this class of compounds. Consequently, the emergence of resistance to these agents may become an increasingly important consideration. There are reports of topoisomerase I mutations derived from cell lines selected for resistance to camptothecin or its derivative, CPT-11. In two of these resistant cell lines, the mutant enzyme has altered topoisomerase I activity with a reduced capacity to mediate camptothecin-induced DNA strand breaks.

MDR ASSOCIATED WITH ALTERED EXPRESSION OF DRUG-METABOLIZING ENZYMES AND DRUG-CONJUGATE EXPORT PUMPS The manner in which cells metabolize cancer drugs and other xenobiotics is often described as three phases of detoxifications (Figure 48-2). While none of these phases are obligatory steps in the metabolism of every drug, the concept illustrated in Figure 48-2 represents a useful framework with which to view cellular detoxification mechanisms. Alterations in any of these three phases can influence the sensitivity or resistance to a particular drug or xenobiotic toxin. Phase I metabolism is mediated by cytochrome P450 mixed-function oxidases. Generally, the drug or xenobiotic is rendered a more electrophilic, reactive intermediate—a process that may enhance toxicity. These metabolites, or the unmodified drug, may then be converted to a less-reactive, presumably less-toxic, form in phase II reactions. Phase II detoxifications include the formation of drug/xenobiotic conjugations with glutathione (GSH), glucuronic acid, or sulfate—reactions that are catalyzed by multiple isozymes each of glutathione S-transferase (GST), uridine diphosphate (UDP)-glucuronosyl transferase, and sulfatase, respectively. Phase III detoxification consists of export of the parent drug/xenobiotic or its metabolites by energy-dependent transmembrane efflux pumps, including MRP family members as described above.

Frequently, in cellular and animal models of drug or xenobiotic resistance, a coordinated downregulation or upregulation of specific phase II drug-conjugating enzymes is observed. Such a programmed cellular stress response offers a versatile, generalized protective mechanism against exposure to a variety of exogenous toxins.

Of the phase II enzymes, the GSTs have been the most extensively studied. GSTs, comprise multiple soluble and membrane-associated
isozymes, which catalyze the conjugation of electrophilic, hydrophobic compounds (R-X) with the thiol, GSH:

\[ R – X + GSH \xrightarrow{\text{GST}} R – SG + H_2O \]

Circumstantial evidence links the increase in specific GST isozymes or bulk GST activity in cells to resistance to alkylating agents and other drugs.\(^{165,164,170-172}\) However, direct evidence that GSTs are responsible for altering drug sensitivities is limited. Another catalytic activity, selenium-independent glutathione peroxidase activity, has been attributed to some isozymes of GST:

\[ R – O – OH + 2 \text{GSH} \xrightarrow{\text{GST}} R – OH + \text{GSSG} + \text{H}_2\text{O} \]

This and other GST-mediated reactions are of interest because of their potential to detoxify oxidative damage to membranes and DNA.

Studies using cell-free preparations of GSTs have identified a limited number of antineoplastic drug substrates of these enzymes. Table 48-5 lists these drugs and other substrates that are possibly associated with drug-mediated oxidative damage. Whether GST levels in tumor cells are sufficient to detoxify antineoplastic drugs to a clinically significant extent is a matter of considerable debate. Several cancer drugs, particularly reactive electrophilic alkylating agents, can form conjugates with glutathione—both spontaneously and in enzyme-catalyzed reactions.\(^{174-181}\) However, despite these catalytic activities, the role of GSTs in drug resistance remains uncertain because of inconsistent results from different laboratories.\(^{172,181-191}\) Indeed, some investigators have found an association between cellular resistance to some antitumor drugs and expression of a particular isozyme of GST, whereas others have found no such association.

The importance of drug/xenobiotic-conjugate transporters for cellular export and detoxification of certain compounds is increasingly appreciated. Conjugation frequently renders the parent drug more hydrophilic and less capable of diffusing the plasma membrane—trapping the drug within the cell. While conjugation with glutathionyl or glucuronosyl groups may render some drugs less toxic, these drug conjugates themselves may retain significant toxicity. For example, the glutathione conjugate formed with cisplatinum is itself toxic and an inhibitor of protein synthesis.\(^{192}\) Moreover, drug conjugates may inhibit their conjugating enzyme(s).\(^{193}\) Thus, the relative resistance of cells expressing drug-metabolizing enzymes may depend on cellular levels of drug conjugate transporters, including the glutathione conjugate transporters.\(^{162,194}\) Indeed, recent results using model cell lines demonstrate that combined expression of specific isozymes of GST with MRP1 is necessary to achieve full protection from the toxicities of the cancer drug chlorambucil,\(^{41}\) or from the carcinogen 4-nitroquinoline 1-oxide.\(^{195}\) In these studies, the expression of either GST or MRP1 alone provided little, if any, protection from toxicity—a finding that illustrates the synergistic interaction of phase II and phase III detoxification processes in the emergence of resistance to some drugs and other xenobiotics.

**Emergence of Refractory Tumors Associated with Multiple Resistance Mechanisms**

The backbone of many treatment protocols designed to circumvent the proliferation of resistant tumor cells is the administration of multiple drugs with different structural properties and mechanisms of action. The approach supposes that if enough carefully selected drugs are delivered at optimal doses and intervals, individual clones of cells resistant to one class of drug will be effectively killed by another drug in the regimen. The rapid appearance of refractory tumors despite an initially favorable cytoreduction response suggests that the emergence of tumor cell clones with multiple resistance is a common clinical occurrence. We have seen how a single genetic change, such as increased P-glycoprotein or altered topoisomerase II, can mediate cross-resistance to several, but not all, useful antineoplastic drugs. Although these mechanisms provide a molecular explanation for broad-spectrum resistance, it is clear that many refractory tumor clones must simultaneously develop multiple resistance mechanisms. These mechanisms may arise from multiple independent genetic changes in single-cell clones or, as suggested by Cadman, from cell-to-cell transfer of genetic information.\(^{196}\)

**Resistance to Anticancer Genotoxic Treatments Related to Suppression of Apoptotic Pathways**

Chemotherapeutic drugs initiate cytotoxicity through their interactions with a variety of molecular targets. For example, epipodophyllotoxins attack topoisomerase II, alkylating agents form adducts with the nucleophilic centers of DNA and proteins, and methotrexate inhibits dihydrofolate reductase, resulting in reduced pyrimidine and purine synthesis. Despite these varied primary targets, most, if not all, cancer drugs effect cell death, at least partially, via downstream events—events that converge upon pathways mediating programmed cell death or apoptosis.

Apoptosis refers to an orderly cellular death program with predictable molecular and morphologic changes, including nuclear pyknosis and fragmentation, internucleosomal endonucleolytic DNA fragmentation, formation of cytoplasmic apoptotic bodies, and plasma membrane changes, such as transposition of phosphatidylserine to the extracellular surface.\(^{197}\) The process is conveniently conceptualized in three phases. First, initiation of apoptosis (eg, secondary to chemotherapy-mediated DNA damage) is characterized by its reversibility. Second, commitment represents the irreversible decision to complete the death program. The commitment phase may involve mitochondrial changes including the permeability phase transition and the release of cytochrome c and apoptosis-inducing factor (AIF)—changes that are hallmarks of apoptosis. Finally, the degradation or execution phase includes downstream events, including DNA fragmentation and morphologic changes. Prior to commitment, apoptosis can be modulated by regulatory elements, such as p53 and the Bcl-2 family proteins.\(^{20,197-199}\) Clearly, such regulation of the apoptotic response can have profound effects on the outcome of chemotherapy and is an area of active investigation germane to drug resistance and sensitivity.

Although apoptosis may be either p53-dependent or independent, frequently the cellular response to DNA damage is regulated by p53.\(^{199}\) As shown in a simplified diagram (Figure 48-3), cancer therapy–induced DNA damage is sensed by p53 by incompletely understood

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**Table 48-5 Some Important Substrates of GSTs Related to Drug Detoxification and Repair of Drug-Mediated Damage**

<table>
<thead>
<tr>
<th>Antineoplastic Drugs</th>
<th>Products of Membrane and DNA Oxidation</th>
</tr>
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<tbody>
<tr>
<td>Nitrogen mustards</td>
<td>Fatty acid hydroperoxides</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>4-Hydroxy alkenals</td>
</tr>
<tr>
<td>Melphalan</td>
<td>DNA hydroperoxides</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
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<tr>
<td>Thiotepa</td>
<td></td>
</tr>
<tr>
<td>Nitrosoureas</td>
<td>1,3-bis(2-chloroethyl)</td>
</tr>
<tr>
<td>Anthracenedione</td>
<td>-1-nitroso urea (BCNU)</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td></td>
</tr>
</tbody>
</table>
mechanisms. Depending on the particular cell type and damage, p53 may then initiate one of two possible pathways: apoptosis or a process of cell-cycle arrest and repair. In cells where the apoptotic pathway dominates, changes that cause dysfunction or deletion of p53 are likely to result in reduced apoptosis in response to DNA damage, leading to relative resistance and cell survival with damage. Indeed, p53 is required for radiation- and etoposide-induced apoptosis in thymocytes, whereas lymphoma cell lines expressing mutant p53 were relatively resistant to DNA-damaging agents. In cells where the p53-dependent cell-cycle arrest and repair response dominates, deletion or mutation of p53 might be expected to result in decreased cell-cycle arrest and repair leading to accumulated DNA damage and hence sensitivity to the chemotherapeutic agent.

The mitogen-activated protein kinase (MAPK)-signaling cascades are involved in the regulation of cellular response to exogenous factors, including geno- and cytotoxic cancer treatments. The extracellular stimulus-regulated kinase (ERK1/2) pathway is implicated in the proliferative response to growth factors. In cells treated with potentially cytotoxic stressors, such as radiation or anticancer drugs, the p38 and stress-activated/c-Jun N-terminal protein kinase (SAPK/JNK) pathways are implicated in mediating cell-cycle arrest or apoptosis. Modulation of these interacting pathways can have a profound effect on whether a cancer cell responds to cytotoxic challenge by activation of apoptosis or by cell-cycle arrest, repair, and hence relative resistance to treatment.

The Bcl-2 family proteins comprise several important regulators of apoptosis. Although their mechanism(s) of action is incompletely known, the balance of expressed antiapoptotic family members (Bcl-2, Bcl-XL, Bcl-w, A1, and Mcl-1) and proapoptotic family members (Bax, Bak, Bad, Bik, and Bid) can influence the relative sensitivity of cells to toxic stressors. Indeed, increased Bcl-2 and its antiapoptotic homologs are associated with increased resistance of lymphoid cells to the cytotoxic effects of corticosteroids, radiation, and DNA damage from chemotherapeutic drugs. It has been proposed that increased levels of antiapoptotic proteins Bcl-2 or Bcl-XL may result in reduced sensitivity to DNA-damaging cancer drugs—a resistance phenotype characterized by cell survival with increased tolerance of DNA damage and genomic instability. This genomic instability may further lead to mutations activating additional resistance mechanisms and conferring more aggressive tumor behavior. Thus, the expression of mutant and wild-type p53, Bcl-2 family members, MAPK family members, and other proteins associated with the control of apoptosis may contribute significantly to the clinical sensitivity of tumor cells. These proteins are the targets of investigational agents that may become important in future strategies to overcome clinical drug resistance.

**RESISTANCE FACTORS UNIQUE TO TUMOR CELLS IN VIVO: HOST-TUMOR INTERACTIONS**

The failure of chemotherapy to eradicate a tumor in vivo despite exquisite sensitivity to drug in vitro might be a result of anatomic or pharmacologic sanctuaries. For example, the failure to deliver adequate amounts of many drugs across blood–brain and blood–testicular barriers probably accounts for the relatively high frequency of acute lymphoblastic leukemia relapse at these sites. In large solid tumors, chemotherapeutic failures are frequently attributed to decreased drug delivery to a tumor that has overgrown its vascular supply. Additionally, development of acidosis and hypoxia in poorly perfused areas of large tumors may interfere with the cytotoxicity of some drugs. Altered prodrug activation by liver or other normal tissues may profoundly influence the efficacy of drugs such as cyclophosphamide.

A report by Teicher and colleagues suggests that tumor-host interactions may influence drug pharmacokinetics and tumor resistance in unexpected ways. In this study, tumor cells selected for cyclophosphamide and cisplatin resistance in vivo were normally sensitive to drugs in vitro. When the tumor cells were reimplanted into nude mice, in vivo drug resistance was restored. These results suggest that resistant tumors may harbor cellular resistance factors that are operative in conjunction with host factors and therefore mediate resistance by altered drug pharmacokinetics in vivo only. If this novel host-dependent mechanism of tumor resistance proves common, these results would provide one explanation for the failure of conventional in vitro testing to predict clinical responsiveness in all cases.

**POTENTIAL CLINICAL APPLICATION OF STRATEGIES TO AVOID OR OVERCOME DRUG RESISTANCE**

Approaches to overcome chemotherapeutic failures include efforts to prevent the emergence of drug resistance (Table 48-6). An appreciation of factors that induce resistance mechanisms may lead to the choice of more efficacious treatment regimens. For example, drugs that may have only sporadic activity against a specific tumor yet are likely to select for cross-resistance to more active agents would be avoided. It is hoped that aggressive combination chemotherapy with non–cross-reacting drugs will eliminate tumor rapidly enough to prevent the selection of tumor cell clones with multiple resistance. Failures of the preventive approach require the incorporation of specific measures aimed at reversing or circumventing drug resistance.

**Table 48-6 Approaches to Overcome or Circumvent Drug Resistance**

| Prevention | Aggressive multiple-agent therapy |
| Correlation of factors that induce resistance mechanisms |
| Circumvention | Drug-screening programs and rational drug design |
| Circumvention of drug uptake defects | Dose escalation |
| Drugs that use alternative transport mechanisms |
| Agents that reverse increased efflux |
| Cofactors that augment drug activation or efficacy |
| Inhibition of drug inactivation |
| Novel treatment modalities |
| Immunotherapy |
| Development of agents that target signalling and apoptotic pathways |
considerable clinical experience in the use of MDR-reversing agents has existed for the treatment of other disorders, these agents have been included in several clinical trials designed to enhance the antitumor activity of conventional cancer drugs in refractory human neoplasms.

While there have been several encouraging studies showing the efficacy of P-glycoprotein/mdr1 inhibitors in murine models of MDR, most promising early clinical trials have been confined to those treating refractory or relapsed hematologic malignancies. Determining the role of MDR in the treatment of solid human tumors has been generally disappointing. Two recent Phase II studies of MDR1 reversal with PSC833 (valspodar), a non-immunosuppressant analog of cyclosporine A, in combination with paclitaxel and doxorubicin in refractory ovarian cancer failed to show a convincing benefit, with complete response rates of 0% and 3%, respectively, and partial response rates of 9% and 12%, respectively.

The largest recent Phase II studies of the potential benefit MDR1 reversal have focused on patients with refractory leukemia. One of these studies, in which 226 AML patients were randomized to receive daunorubicin in a 72-h infusion with or without concomitant infusion of cyclosporine A, showed that the addition of cyclosporine A improved relapse-free survival (34% versus 9% at 2 years; \( p = 0.031 \)) and overall survival (22% versus 12%; \( p = 0.046 \)). It is of interest in this study that cyclosporine A did not improve the remission rate, and that survival improved with increasing daunorubicin concentrations in the cyclosporine-treated patients but not in the controls, suggesting that the addition of cyclosporine A enhances the cytotoxicity of daunorubicin and eliminated resistant clones. This prospective clinical study of infusional cyclosporine A and daunorubicin in AML by List and coworkers demonstrated for the first time a clear clinical benefit from all of preclinical studies of drug resistance.

In addition to their actions on P-glycoprotein–positive tumor cells, MDR-reversing agents can have profound effects on the pharmacokinetics and pharmacodynamics of cytotoxic drugs associated with MDR. As noted above, marked increases in the area under the curve levels, decreased renal and nonrenal clearances, and increased volumes of distribution of etoposide have been observed in patients concomitantly treated with cyclosporin A. The reason for these effects is unknown, but it is suggested they are due to the action of cyclosporins on normal tissues—such as renal, biliary, and endothelial cells—possibly via cyclosporin interactions with the P-glycoprotein resident within these normal tissues. Toxicities of MDR-associated drugs, such as myelosuppression, may be enhanced when administered with reversing agents. These toxicities necessitate appropriate reduction in the dosage of cytotoxic drugs when they are used in combination with cyclosporins. Because P-glycoprotein is found at high levels in central nervous system (CNS) endothelium and contributes to the blood-brain barrier, concomitant administration of MDR-associated chemotherapeutic drugs and P-glycoprotein inhibitors may also enhance neurotoxicities. These pharmacologic issues must be carefully considered in future clinical trials.

Alternative strategies for reversing P-glycoprotein–mediated MDR include the use of monoclonal antibodies directed against extracellular epitopes of P-glycoprotein, anti–P-glycoprotein antibody–toxin conjugates that target P-glycoprotein expressing MDR tumor cells, or anti–P-glycoprotein antibodies engineered to recruit activated T-lymphocytes for the cytolysis of P-glycoprotein expressing tumor cells. Other approaches to reversing P-glycoprotein–mediated MDR include antisense and ribosome nucleotides directed against MDR mRNA. It remains to be determined whether considerable obstacles to the clinical application of inhibitory polynucleotides can be overcome. All the approaches to inhibiting or targeting P-glycoprotein expression may be limited due to the normal expression of this protein pump in normal tissues, including kidney, liver, colon, and endothelial cells of the CNS. In addition, encapsulating drugs in liposomes may be an effective means of avoiding MDR1 and other membrane-bound efflux pumps.

Similar approaches for reversing MRP family–mediated MDR are possible. A number of compounds inhibit MRPI-mediated efflux activity, including the organic acids probenecid and sulfinpyrazone, the LTD4 antagonist MK571, cyclosporin A, and the cyclosporin derivative, PSC 833. Finally, MRPI-mediated transport of some drugs is dependent on intracellular glutathione either as a noncovalent cofactor or as a moiety covalently linked, nonenzymatically or by GST, to some electrophilic anticancer drugs. Thiol depletion of tumor cell glutathione or inhibition of GST (see section on Resistance to Free Radical–Medicated Drug Cytotoxicity) offer potential strategies for secondarily reversing MRPI-mediated drug resistance. Some substrates of MRPI are glucuronide and sulfate derivatives of the parent drug. Selective inhibition of tumor cell UDP-glucuronosyl transferases or sulfotransferases could also represent a future avenue for secondary reversal of MRPI-associated drug resistance.

TOXICITY P-OISES As discussed above, resistance to topoisomerase II poisons may occur as a consequence of P-glycoprotein overexpression or altered topoisomerase II activities. However, neither of these mechanisms will necessarily result in cross-resistance to all the topoisomerase II–directed drugs listed in Table 718-3. For example, resistance to epipodophyllotoxins and anthracyclines on the basis of increased P-glycoprotein is not usually associated with resistance to the acridine derivative, amsacrine. Conversely, resistance to amsacrine and other intercalating drugs caused by alterations in topoisomerase II protein is not always associated with resistance to the nonintercalating, epipodophyllotoxin class of topoisomerase II poisons. Therefore, these data derived from in vitro studies suggest a rationale for administering an alternative class of topoisomerase II poison in selected cases of clinical resistance to another class of topoisomerase II–directed drug. Additionally, tumor cells resistant to classic topoisomerase II poisons (Table 718-4) frequently retain sensitivity to the cytotoxities of the novel class of topoisomerase II–catalytic inhibitors (fostriecin, mepacrine, aclacinomycin, and bis [2,6-dioxopiperazines]). This class of topoisomerase-directed drug offers an alternative for the treatment of topoisomerase poison–resistant tumors. Finally, structural analogs of parent topoisomerase II poisons may overcome resistance based on altered topoisomerase II.

Resistance to Free Radical–Mediated Drug Cytotoxicity Several pathways may contribute to protection of tumor cells from anthracycline-mediated free radical damage. First, superoxide anion formation is limited in poorly vascularized, relatively hypoxic tissues, such as may exist in the centers of large solid tumors. Second, increased intracellular levels of catalase and glutathione peroxidase (GSHPx) can deplete hydrogen peroxide, thus reducing the formation of toxic hydroxyl radicals. Indeed, in comparing parental and MDR MCF7 cells, Sinha and coworkers reported an association between increased GSHPx activity and reduced doxorubicin-stimulated hydroxyl radical formation. Furthermore, lowering GSHPx activity by depleting the enzyme’s cosubstrate, GSH, resulted in enhanced doxorubicin-dependent free radical formation and cytotoxicity. Additionally, Kramer and colleagues found that GSH depletion with buthionine sulfoximine (BSO) could partially restore the doxorubicin sensitivity of MDR MCF7 cells, presumably by interfering with GSH-dependent reactions, including those catalyzed by GSHPx. While these results are consistent with the importance of hydrogen peroxide and hydroxyl radical formation in anthracycline cytotoxicity in MCF-7 cells, other investigators have noted that increased catalase, GSH, and GSHP levels are not always protective of some cells from doxorubicin-mediated damage.

The relative importance of free radical generation in tumor cell kill is unknown, and the protective mechanisms outlined above are speculative. Nevertheless, the GSH-dependent detoxification pathways are of particular interest as they are subject to pharmacologic manipulation. GSHPxs and GST activities can be secondarily reduced by depleting tissue GSH with BSO treatment. Furthermore, the activity of GSTs can be inhibited by the administration of competitive substrates, such as ethacrynic acid. Such clinical manipulations may enhance tumoricidal activity of doxorubicin but
must be viewed cautiously as they may also potentiate drug toxicity toward normal tissues.

**Alkylation Agents and Platinum Compounds** Resistance to alkylation agents and platinum compounds can be described by at least three broad mechanistic categories, including decreased drug accumulation, increased drug inactivation, and enhanced repair of DNA damage.\(^{17,263–265}\) Additionally, the nature of the tumor cells' response to alkylation agent damage—whether primarily apoptosis, repair, or survival with damage—will contribute significantly to the outcome of alkylation agent treatment. Preclinical studies have indicated that all these mechanisms may be circumvented, at least partially, by pharmacologic manipulations. Reactions of electrophilic alkylation agents with thiol-containing compounds represent relatively general mechanisms of antineoplastic inactivation or detoxification. For example, GSH forms conjugate with a variety of alkylation agents in both nonenzymatic and in GST-dependent reactions. Table 48-5 lists some of the compounds whose conjugation with GSH is catalyzed by GSTs in vitro.\(^{172}\) Several laboratories have demonstrated an association between increased bulk GST levels or specific GST isozymes with resistance to drugs such as nitrosoureas,\(^{266}\) chlorambucil, and other nitrogen mustards.\(^{183,187,267–269}\) Additionally, increased GSH levels correlate with resistance to alkylation agents and cisplatin.\(^{270,271}\) While the electrophilic cisplatin conjugate can react directly with GSH, it is unknown whether GSTs can catalyze this reaction. This issue is unresolved because of conflicting results that show a correlation between elevated expression of the pi isozyme of GST and resistance to cisplatin in some cells.\(^{272,273}\) but not others.\(^{186}\) Perhaps more relevant to the issue of cisplatin resistance is the finding that glutathionyl-platinum complexes, which are themselves toxic, are exported by an ATP-dependent pump probably identical to one of the glutathione conjugate pumps described previously.\(^{192}\) Thus, these drug exporters should be considered in the design of treatments and formulation of strategies to enhance cisplatin efficacy.

The correlations between GSH or GST levels and drug resistance are variable. Indeed, some investigators have been unable to demonstrate a relationship between the overexpression of multiple isozymes of GST and antineoplastic resistance.\(^{64,185,186,190}\) In studies that have compared paired parental and resistant cell lines, the magnitude of alkylation agent resistance associated with increased GST activity is often modest. As noted above, for some drugs such as chlorambucil, the coexpression of a glutathione conjugate efflux transporter appears to be required for the emergence of GST-mediated resistance in the MCF7 cell model system.\(^{31}\) While the clinical importance of GST and GSH in alkylating resistance is, accordingly, debated, existing preclinical data has prompted Phase I trials using GST inhibitors, or the GSH synthesis inhibitor BSO, in conjunct with alkylating agents. Three Phase I trials have been reported with the combination of BSO and melphalan. These trials demonstrate that it is possible to deplete glutathione levels by coadministration of BSO with alkylating agent chemotherapy. However, no Phase II studies have yet been reported to demonstrate the antitumor efficacy of this approach.

Aldehyde dehydrogenase is another drug-metabolizing enzyme that is linked to cyclophosphamide-derivative resistance in murine and human models of drug resistance.\(^{274–276}\) This enzyme converts aldophosphamide, a metabolite of cyclophosphamide, to the inactive compound, carboxyphosphamide, thereby preventing the decomposition of aldophosphamide to its cytotoxic derivative, phosphoramide mustard. Increased expression of aldehyde dehydrogenase is associated with resistance to cyclophosphamide in vitro. Whether inhibitors of aldehyde dehydrogenase, such as disulfiram and diethylaminobenzaldehyde, can be used therapeutically to enhance the antitumor effect of cyclophosphamide without undue host toxicity remains to be explored.

Cisplatin toxicity is thought to be mediated primarily by the formation of lethal intranastad DNA cross-links. Several reports suggest that either increased DNA repair or tolerance of DNA damage is associated with resistance to this compound. In a murine leukemia model, cells selected for cisplatin resistance showed enhanced ability to repair cisplatin-induced intranastad DNA cross-links.\(^{277,278}\) Aphidicolin can inhibit an enzyme implicated in DNA repair, DNA polymerase alpha. Treatment of ovarian carcinoma cells with aphidicolin potentiated the toxicity of cisplatin in resistant but not sensitive cells.\(^{279}\) These results suggest that the coadministration of DNA polymerase alpha inhibitors with cisplatin may be useful in overcoming cisplatin resistance. Also implicated in platinum sensitivity and resistance are alterations in mismatch repair of regulators of apoptosis, such as Bcl-2, Bax, p21, or p53.\(^{17}\) Modulation of these pathways by therapeutic agents now in development represents an emerging strategy for overcoming resistance to platinum and other alkylating compounds.

**Antimetabolites** The antimetabolites are a clinically important group of cancer drugs used in the treatment of a variety of solid tumors and hematologic malignancies. The cytotoxicities of the antimetabolites stem from their ability to interfere with key enzymatic steps in nucleic acid metabolism. The discussion which follows concerns three particularly well-studied compounds, the antifolate methotrexate (MTX) and the pyrimidine analogs 5-fluorouracil (5-FU) and cytosine arabinoside (ara-C, 1-β-D-arabinofuranosylcytosine, cytarabine). Strategies designed to overcome the multiple described mechanisms of cellular resistance to these compounds include dose escalation, pharmacologic manipulation of drug metabolism, and rational design of new antimetabolites.\(^{280}\)

MTX, the clinically important antifolate, displays significant tumoricidal activity against a variety of human neoplasms, such as acute leukemia, osteogenic sarcoma, choriocarcinoma, breast cancer, head and neck cancers, and others.\(^{281}\) Consideration of MTX metabolism and sites of action (Figure 48-4) serves as the basis for understanding mechanisms of methotrexate resistance. Following uptake by the folate transport systems, MTX can bind avidly to and inhibit its primary enzyme target, dihydrofolate reductase (DHFR). In the presence of adequate thymidylate synthase activity, inhibition of DHFR results in depletion of the reduced folate pools essential for thymidylate and de novo purine synthesis. The cytotoxicity of MTX is significantly influenced by intracellular polyglutamation. MTX polyglutamates are retained preferentially by cells and bind more effectively to DHFR. Additionally, these polyglutamyl derivatives can inhibit other folate-dependent enzymes, including thymidylate synthase and 5-aminomimidazole-4-carboxamide ribonucleoside (AICAR) transformylase, enzymes involved in thymidylate and de novo purine synthesis, respectively. Therefore, resistance to MTX can result from a number of alternative mechanisms, including (1) reduced MTX

![Figure 48-4 Methotrexate metabolism and toxicity. DHFR = dihydrofolate reductase; FH\textsubscript{2} = dihydrofolate; FH\textsubscript{4} = tetrahydrofolate; 5,10 MEFH\textsubscript{4} = 5,10-methylene tetrahydrofolate; MTX = methotrexate; MTX-(glun) = pglutamate methotrexate; TS = thymidylate synthase.](image)
uptake via a defective folate transport system, such as decreased expression of the reduced folate carrier or of the folate receptors; (2) increased export via MRP family proteins or other exporters of polyglutamatable antifolates; (3) reduced polyglutamation leading to decreased drug retention as well as reduced inhibition of thymidylate synthase and AICAR transformylase; and (4) either elevated levels of DHFR or reduced affinity of DHFR for MTX.

The use of high-dose MTX (HDMTX) with subsequent rescue of normal tissues by administration of the reduced folate, leucovorin (N5-formyl tetrahydrofolate) has been advocated as an approach that could theoretically circumvent most mechanisms of MTX resistance. At high systemic drug concentrations, cytotoxic levels can be achieved by passive diffusion of drug into transport-defective resistant cells. Furthermore, prolonged exposure of cells to high extracellular concentrations of drug can maintain cytotoxic intracellular drug levels in the face of a drug retention defect secondary to decreased polyglutamation. Finally, increased intracellular MTX delivered by HDMTX therapy can saturate DHFR in cells whose resistance is a result of amplification of the DHFR gene or of lowered affinity of DHFR for MTX. Although HDMTX is of proven value in the treatment of ALL and perhaps of osteogenic sarcoma, the rationale for the use of this modality in the treatment of other cancers was recently questioned. Indeed, some tumors, as well as normal tissues, are rescued from HDMTX toxicity by leucovorin. In these and other cases, the use of HDMTX with leucovorin rescue offers no therapeutic advantage over regimens that use conventional MTX and leucovorin rescue offers no therapeutic advantage over regimens that use conventional MTX doses. While early studies suggested that HDMTX improved response rates to chemotherapy of osteogenic sarcoma, the contribution of HDMTX therapy to the success of recent multiagent adjuvant protocols is unclear. In contrast, HDMTX is indisputably efficacious in the treatment of ALL. The success of HDMTX in this setting is probably a result of the penetration of drug across anatomic and pharmacologic barriers into tumor sanctuaries, such as testes, and, at very high MTX doses, the central nervous system.

In an effort to improve drug efficacy, other inhibitors of DHFR, such as trimetrexate and piritecumiz, were developed. These lipid-soluble drugs are taken up by cells independently of the folate-carrier system; consequently their use might obviate transport-mediated antifolate resistance. However, cells that are resistant to MTX on the basis of amplified DHFR will be cross-resistant to trimetrexate. The utility of trimetrexate is further limited by the association of classic MDR with cross-resistance to trimetrexate. Cells can also overcome antifolate toxicity by increasing the salvage of nucleoside precursors. One mechanism of overcoming these mechanisms of resistance is the concomitant administration of nucleoside transport inhibitor nitrobenzylmercaptopurine riboside (NBMPR) with antifolate drugs, which enhances antifolate cytotoxicity in rodent models.

The pyrimidine base, 5-FU and its deoxynucleoside metabolite, 5-fluoro-2'-deoxyuridine (FdUrd) have been used in the treatment of gastrointestinal tumors, breast cancer, head and neck cancer, and some other malignancies. The metabolism of 5-FU is complex and is partially shown in Figure 48-5. The best characterized mechanism of fluoropyrimidine cytotoxicity involves the inhibition of thymidylate synthase by 5-fluoro-2'-deoxyuridine monophosphate (FdUMP). Additionally, the incorporation of the metabolite, 5-fluorouridine triphosphate (FUTP) into RNA correlates with cytotoxicity in some systems. While 5-fluoro-2'-deoxyuridine triphosphate (FdUTP) can be incorporated into DNA, the relationship between this process and the cytotoxic activity of fluoropyrimidines remains undetermined. Resistance to 5-FU may be conferred by alterations in enzymes involved in fluoropyrimidine metabolism, particularly those enzymes associated with the conversion of 5-FU to the thymidylate synthase inhibitor, FdUMP. Furthermore, changes in thymidylate synthase level or its affinity for FdUMP are associated with 5-FU resistance.

Several strategies to improve fluoropyrimidine efficacy and overcome resistance have been advanced. It has been suggested that tumor cell killing may be improved by prolonged or continuous exposure to drug. Other studies advocate the coadministration of 5-FU with the reduced folate leucovorin. The efficacy of this combination stems from leucovorin-dependent increases in intracellular 5,10-methylene tetrahydrofolate (5,10-MTHF), a cofactor that stabilizes the FdUMP-thymidylate synthase inhibitor complex. Synergy between 5-FU and other agents, which might be exploited clinically, has also been studied. For example, pretreatment of cells with methotrexate enhances the toxicity of 5-FU subsequently administered. Such pretreatment with methotrexate, an inhibitor of de novo purine synthesis (discussed above), increases the level of phosphoribosyl pyrophosphate (PRPP). Thus, the expanded pool of PRPP is available for conversion of 5-FU to FUMP and FUTP (see Figure 48-5). It has been suggested that the increased incorporation of FUTP into RNA that results is responsible for the improved cytotoxicity. The inhibitor of de novo pyrimidine synthesis, N-phosphonacetyl-L-aspartic acid (PALA), has been used with 5-FU in an effort to reduce pyrimidine metabolites that compete for the targets of fluoropyrimidine toxicity. Finally, the synergistic interaction between interferon and halogenated pyrimidines has been investigated.

Ara-C is an important nucleoside antineoplastic agent effective in the treatment of acute leukemias. Figure 48-6 presents the metabolism and mechanism of cytotoxicity of ara-C. Following its uptake by the nucleoside transport system, ara-C is activated by a series of kinases to ara-CTP, a substrate of DNA polymerase that is incorporated into nascent DNA, causing premature chain termination and ultimately cell death. The rate-limiting step in ara-C activation is the S-phase specific reaction catalyzed by deoxycytidine kinase. The cytotoxic compound, ara-CTP or its precursors (ara-CMP and ara-CDP) can be catabolized by phosphatases or they (ara-C and ara-CMP) can be inactivated by deaminases. Several mechanisms of cancer cell resistance to ara-C have been demonstrated, including, but not confined to, the following. Because ara-C activation is cell-cycle dependent,
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