

MECHANISMS OF CANCER DRUG RESISTANCE

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■ **Abstract** The design of cancer chemotherapy has become increasingly sophisticated, yet there is no cancer treatment that is 100% effective against disseminated cancer. Resistance to treatment with anticancer drugs results from a variety of factors including individual variations in patients and somatic cell genetic differences in tumors, even those from the same tissue of origin. Frequently resistance is intrinsic to the cancer, but as therapy becomes more and more effective, acquired resistance has also become common. The most common reason for acquisition of resistance to a broad range of anticancer drugs is expression of one or more energy-dependent transporters that detect and eject anticancer drugs from cells, but other mechanisms of resistance including insensitivity to drug-induced apoptosis and induction of drug-detoxifying mechanisms probably play an important role in acquired anticancer drug resistance. Studies on mechanisms of cancer drug resistance have yielded important information about how to circumvent this resistance to improve cancer chemotherapy and have implications for pharmacokinetics of many commonly used drugs.

INTRODUCTION

The treatment of disseminated cancer has become increasingly aimed at molecular targets derived from studies of the oncogenes and tumor suppressors known to be involved in the development of human cancers (1). This increase in specificity of cancer treatment, from the use of general cytotoxic agents such as nitrogen mustard in the 1940s, to the development of natural-product anticancer drugs in the 1960s such as *Vinca* alkaloids and anthracyclines, which are more cytotoxic to cancer cells than normal cells, to the use of specific monoclonal antibodies (2) and immunotoxins (3) targeted to cell surface receptors and specific agents that inactivate kinases in growth-promoting pathways (4), has improved the response rate in cancer and reduced side effects of anticancer treatment but has not yet resulted in cure of the majority of patients with metastatic disease. A study of the mechanisms by which cancers elude treatment has yielded a wealth of information about why these therapies fail and is beginning to yield valuable information about how to circumvent drug resistance in cancer cells and/or design agents that are not subject to the usual means of resistance.

HOW DO CANCER CELLS ELUDE CHEMOTHERAPY?

Failure of a patient's cancer to respond to a specific therapy can result from one of two general causes: host factors and specific genetic or epigenetic alterations in the cancer cells. Host factors include poor absorption or rapid metabolism or excretion of a drug, resulting in low serum levels; poor tolerance to effects of a drug, especially in elderly patients, resulting in a need to reduce doses below optimal levels; inability to deliver a drug to the site of a tumor, as could occur with bulky tumors or with biological agents of high molecular weight and low tissue penetration such as monoclonal antibodies and immunotoxins (5); and various alterations in the host-tumor environment that affect response of the tumor including local metabolism of a drug by nontumor cells, unusual features of the tumor blood supply that may affect transit time of drugs within tumors and the way in which cells in a cancer interact with each other and with interstitial cells from the host (6).

To paraphrase Tolstoy in the opening lines of *Anna Karenina*, normal cells are all alike in their response to drugs, but cancer cells each respond in their own way. Each cancer cell from a given patient has a different genetic make-up depending not only on the tissue of origin but also on the pattern of activation of oncogenes and inactivation of tumor suppressors as well as random variations in gene expression resulting from the "mutator" phenotype of most cancers. As a result, every cancer expresses a different array of drug-resistance genes, and cells within a cancer, even though clonally derived, exhibit an enormous amount of heterogeneity with respect to drug resistance. In addition, even if tumors are not intrinsically resistant to a specific anticancer treatment, this genetic and epigenetic heterogeneity in the face of the powerful selection imposed by potent anticancer drugs results in overgrowth of drug-resistant variants and the rapid acquisition of drug resistance by many cancers.

For the past 40 years, researchers have been tabulating the various mechanisms by which cancer cells grown in tissue culture become resistant to anticancer drugs (Figure 1). Some of these mechanisms, such as loss of a cell surface receptor or transporter for a drug, specific metabolism of a drug, or alteration by mutation of the specific target of a drug, all of which occur for antifolates such as methotrexate (7), result in resistance to only a small number of related drugs. In such cases, use of multiple drugs with different mechanisms of entry into cells and different cellular targets allows for effective chemotherapy and high cure rates. All too often, however, cells express mechanisms of resistance that confer simultaneous resistance to many different structurally and functionally unrelated drugs. This phenomenon, known as multidrug resistance (8), can result from changes that limit accumulation of drugs within cells by limiting uptake, enhancing efflux, or affecting membrane lipids such as ceramide (9). These changes block (a) the programmed cell death (apoptosis) that is activated by most anticancer drugs (10), (b) activation of general response mechanisms that detoxify drugs and repair damage to DNA (11), and (c) alterations in the cell cycle and checkpoints that render cells relatively

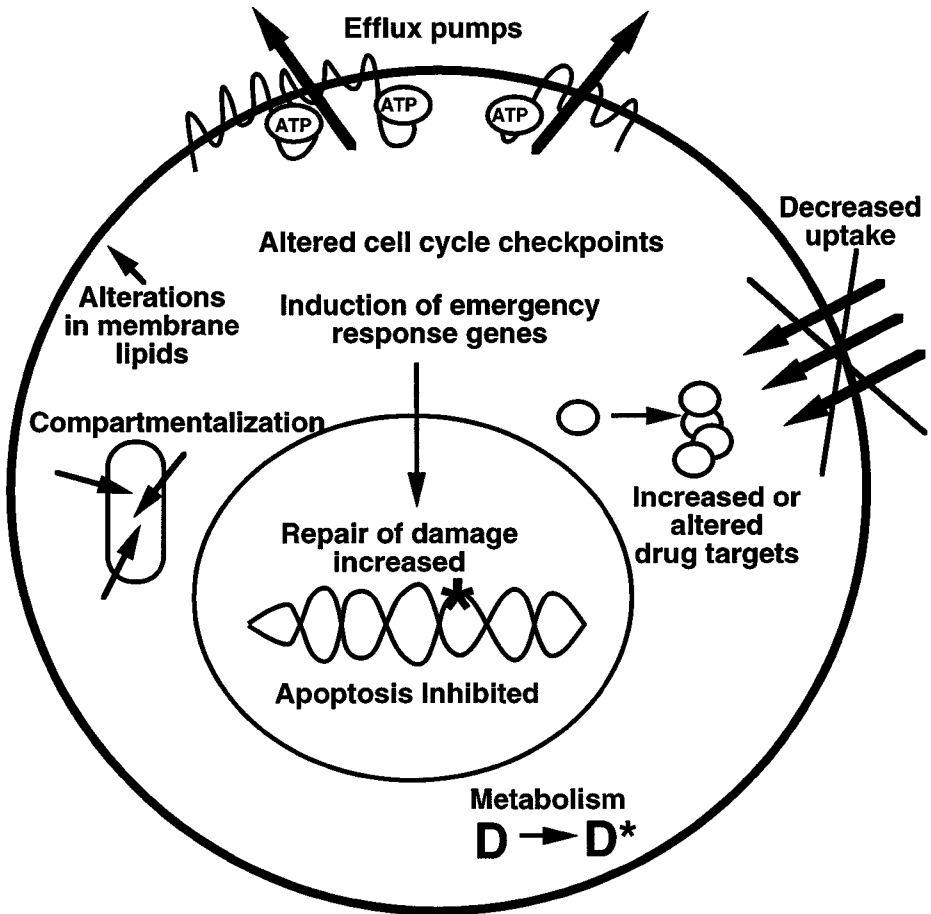


Figure 1 This cartoon summarizes many of the ways in which cultured cancer cells have been shown to become resistant to cytotoxic anticancer drugs. The efflux pumps shown schematically at the plasma membrane include MDR1, MRP family members, and MXR (ABC G2), which is presumed to function as a dimer.

resistant to the cytotoxic effects of drugs on cancer cells. Expression of a major vault protein, termed lung resistance-related protein (LRP), which may regulate nuclear entry of drugs, has also been described in multidrug resistance (11a).

Among these mechanisms, we know the most about those that alter accumulation of drugs within cells (for reviews, see 12, 13). This accumulation results from a balance between drug entry and exit mechanisms. Drugs enter cells in various ways (Figure 2). Each of these mechanisms of entry has been determined to have physiological significance based on detailed uptake studies and on the existence of resistant mutants in which defects in these pathways have been observed.

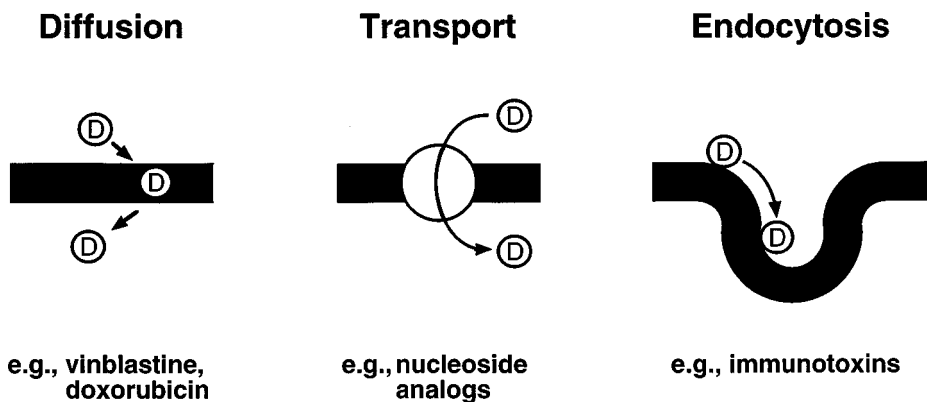


Figure 2 Ways in which drugs can get into cells. Examples are given for the three major routes: diffusion across the plasma membrane, piggy-backing onto a receptor or transporter, and endocytosis.

The remainder of this review focuses on the mechanisms of multidrug resistance resulting from alterations in the pathways of drug uptake or efflux from the cell.

MECHANISMS OF DRUG RESISTANCE THAT INCREASE DRUG EFFLUX FROM CANCER CELLS

It came as something of a surprise that the major mechanism of multidrug resistance in cultured cancer cells was the expression of an energy-dependent drug efflux pump, known alternatively as P-glycoprotein (P-gp) or the multidrug transporter (14, 15). This efflux pump, the product of the *MDR1* gene in the human (16) and the product of two different related genes, *mdr1a* and *mdr1b* in the mouse (17, 18), was one of the first members described of a large family of ATP-dependent transporters known as the ATP-binding cassette (ABC) family (19). Every living organism has encoded within its genome many members of this family, and they appear to be involved not only in efflux of drugs but in moving nutrients and other biologically important molecules into, out of, and across plasma membranes and intracellular membranes in cells. P-gp is widely expressed in many human cancers, including cancers of the gastrointestinal (GI) tract (small and large intestine, liver cancer, and pancreatic cancer), cancers of the hematopoietic system (myeloma, lymphoma, leukemia), cancers of the genitourinary system (kidney, ovary, testicle), and childhood cancers (neuroblastoma, fibrosarcoma) (20). The human gene most closely related to *MDR1* is *MDR2*, a phosphatidylcholine transporter expressed in liver whose defect results in inability to form bile and progressive cirrhosis (21).

P-gp, the human *MDR1* gene product and one of 48 known ABC transporters in the human, is a 170,000-dalton-molecular weight phosphoglycoprotein




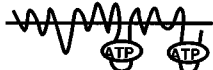


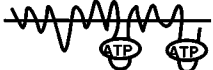

Common Names	Official Name	Structure	Substrates	Normal location
P-gp, MDR1	ABC B1		Neutral and cationic organic compounds	intestine, liver, kidney, blood-brain barrier
MRP1	ABC C1		GS-X and other conjugates, organic anions	widespread
MRP2, cMOAT	ABC C2		GS-X and other conjugates, organic anions	liver, kidney, intestine
MRP3, MOAT-D	ABC C3		GS-X conjugates, anti-folates, bile acids, etoposide,	pancreas, kidney, intestine, liver, adrenal
MRP4, MOAT-B	ABC C4		nucleoside analogs, methotrexate	prostate, testis, ovary, intestine, pancreas, lung
MRP5, MOAT-C	ABC C5		nucleoside analogs, cyclic nucleotides, organic anions	widespread
MRP6, MOAT-E	ABC C6		anionic cyclic pentapeptide	liver, kidney
MXR, BCRP, ABC-P	ABC G2		anthracyclines, mitoxantrone	placenta, intestine, breast, liver

Figure 3 ABC transporters with known drug substrates. Curved lines represent transmembrane domains, and the ATP in the ovals represents the ATP-binding cassettes in these ABC transporters. GS-X represents glutathione conjugates of drugs.

consisting of two ATP binding cassettes and two transmembrane regions, each of which contains six transmembrane domains (16) (Figure 3). P-gp can detect and bind a large variety of hydrophobic natural-product drugs as they enter the plasma membrane. These drugs include many of the commonly used natural-product anticancer drugs such as doxorubicin and daunorubicin, vinblastine and vincristine, and taxol, as well as many commonly used pharmaceuticals ranging from antiarrhythmics and antihistamines to cholesterol-lowering statins (22) and HIV protease inhibitors (23). Binding of these drugs results in activation of one of the ATP-binding domains, and the hydrolysis of ATP causes a major change in the shape of P-gp, which results in release of the drug into the extracellular space (24). Hydrolysis of a second molecule of ATP is needed to restore the transporter to its original state so that it can repeat the cycle of drug binding and release (25, 26). Although the detailed mechanism of action of other ABC transporters is not known, it is presumed that the ATP binding cassette acts as the engine for the transport mediated by members of this large family of transporters.

Because of the promiscuity with which P-gp binds electrically neutral and positively charged hydrophobic drugs within the plasma membrane, many different anticancer drugs and other drugs in common use are substrates for this transport system. Any drug that interacts with the substrate-binding region of P-gp is likely to be a competitive inhibitor of the binding of other drugs. Because of the large size and complex structure of P-gp drug-binding region(s), not all inhibitors have equal potency against all substrates. Active development of potent, specific inhibitors of P-gp is underway as a means to reverse multidrug resistance in cancers, and some have shown activity in sensitizing drug-resistant cancers in patients (see below).

After the discovery of P-gp and the demonstration of its widespread expression in many human cancers, it was found that many multidrug-resistant cancers, such as lung cancers, rarely express P-gp. Using a multidrug-resistant lung cancer cell line as a model system, Deeley and Cole and colleagues cloned another ABC family member, known as *MRP1* (for multidrug resistance associated protein 1) and showed that it had a broad spectrum of anticancer drug transport activity (27) (Figure 3). *MRP1*, unlike *MDR1*, transports negatively charged natural-product drugs and drugs that have been modified by glutathione, conjugation, glucosylation, sulfation, and glucuronylation. In some cases cotransport of glutathione with positively charged drugs such as vinblastine can occur. *MRP1* is also widely expressed in many human tissues and cancers (12).

The discovery of *MRP1* led to a search for other members of this family, resulting in the discovery of a total of 9 or 10 *MRP* genes, at least 6 of which have been characterized enough to indicate that they transport anticancer and antiviral compounds (12, 28–32) (Figure 3). Many of these appear to transport drugs potentially important for the treatment of cancer, and their role in conferring drug resistance on cancer cells is under active investigation.

A third ABC transporter for anticancer drugs has been called *MXR*, *BCRP*, or *ABC-P* (33–35) (Figure 3). It was found to be overexpressed in cells selected for resistance to mitoxantrone or anthracyclines. Unlike *MDR1* and the *MRP* family members, it only has one region with six transmembrane domains and a single ATP-binding cassette (see Figure 1) but is presumed to function as a dimer. Recent evidence suggests that the wild-type form of *MXR* shows a narrower range of substrates for drug transport than a mutant form of the protein in which the amino acid threonine or glycine is substituted for arginine at position 482, which was isolated in the early studies (36). The role of *MXR* in clinical drug resistance remains to be determined.

Three additional ABC transporters have been implicated in drug transport of potential significance to cancer, including the *MDR2* gene product (37), a protein named *SPGP* (sister of P-gp) (38), and *ABC A2* (39), but these data are too preliminary to include in our figure. It is also possible that other members of the ABC transporter family in addition to the *MDR*, *MRP*, and *MXR* family members are involved in clinical cancer drug resistance or in drug transport in the human. Based on the known DNA sequences of these genes, efforts to explore their patterns of expression and determine their function are under way. One approach is to

examine intrinsically resistant cancers and those that have acquired resistance to anticancer drugs and determine whether expression of other ABC transporters occurs commonly.

DRUG RESISTANCE DUE TO REDUCED UPTAKE OF DRUGS

As illustrated in Figure 2, specific and nonspecific uptake of water-soluble drugs can occur across the plasma membrane based on piggy-backing of these drugs on known transporters involved in uptake of nutrients and other essential low molecular weight molecules and by the process of endocytosis, either receptor-mediated or nonspecific. The latter process has been termed pinocytosis and refers to the general means by which cells “drink” extracellular fluids and internalize the variety of compounds that may be dissolved in the extracellular fluid.

Selection of cells for resistance to drugs that enter cells via receptors or transporters can result in mutations that eliminate or modify these cell surface molecules. For example, resistance to toxic folate analogs such as methotrexate commonly occurs by mutation of one or both of the folate transporters (folate binding protein, and/or the reduced folate transporter) (7). Resistance to nucleoside analogs has been described as a result of mutation of specific nucleoside transporters, etc. In general, these mechanisms of resistance are specific for these nutrient analogs and structurally related compounds.

Very few drugs enter cells by endocytosis. However, some of the newer anti-cancer agents, such as immunotoxins that bind to cell surface receptors, cannot kill cells unless they are internalized (3). They are generally internalized via receptor-mediated endocytosis. Cancer cell mutants that have defective endocytosis are resistant to both toxins and immunotoxins (40).

Cisplatin is commonly used to treat cancers such as head and neck cancer, testicular cancer, ovarian cancer, and other solid tumors. It is not known with certainty how cisplatin, a water-soluble compound, enters cells (41). Many different cisplatin-resistant cancer cell lines have been isolated in the laboratory. These exhibit a variety of mechanisms of resistance, but reduced accumulation of the drug is commonly seen, as is cross-resistance to methotrexate, heavy metals such as arsenite and arsenate, cadmium and mercury, and resistance to some nucleoside analogs. We have recently shown that cisplatin-resistant cells demonstrating this pattern of cross-resistance and reduced accumulation of the drug have a pleiotropic defect resulting in reduced plasma membrane receptors and transporters and reduced endocytosis (42). In these cells there is no evidence for an energy-dependent efflux pump for cisplatin (43), which has been described by other researchers in different cell lines. These results suggest that cisplatin may enter cells via receptors and/or via endocytosis. Whether clinical resistance to cisplatin by this mechanism also occurs, with attendant cross-resistance to methotrexate and nucleoside analogs, remains to be determined.

CLINICAL RELEVANCE OF LABORATORY STUDIES ON CANCER DRUG RESISTANCE

As noted, it is possible to demonstrate the presence of several different drug efflux pumps in human cancers. Evidence that ABC transporters, especially P-gp, play a significant role in clinical drug resistance has been reviewed extensively and can be summarized as follows: (a) Levels of expression of P-gp in many different tumors are high enough to confer significant drug resistance, and the presence of P-gp correlates with drug resistance in several different cancers (20); (b) acquisition of drug resistance after chemotherapy is associated with increased P-gp levels (20), and this increased expression occurs via specific molecular mechanisms, such as gene rearrangement and selection of cells showing these rearrangements (44), which argues that the P-gp-expressing cells have a selective advantage; (c) acute induction of P-gp has been observed in human tumors following exposure *in vivo* to doxorubicin (45); (d) in early clinical trials testing P-gp modulation in acute leukemia, cells that survived chemotherapy in the presence of modulators, resulting in clinical relapse, had reduced expression of P-gp (46, 47); and (e) expression of P-gp in some tumors predicts poor response to chemotherapy with drugs that are transported by P-gp (48).

This evidence has been used to support the introduction of various P-gp inhibitors into the clinic, and many studies using such inhibitors have been reported, with more in progress. The first studies were performed with modulators approved for clinical uses other than inhibition of P-gp. Subsequent studies were performed with "second generation" modulators, compounds with somewhat increased potency but still limited by toxicity. Recently, a new generation of inhibitors has reached clinical testing. These "third generation" inhibitors promise to be non-toxic, more specific, and more potent than the earlier inhibitors used in trials of P-gp modulation. These compounds include XR9576, R101933, Biricodar (VX710), and LY335979.

In studies reported to date, with first generation modulators and with dexverapamil, dextriguldipine, and the cyclosporin D analogue Valspodar (PSC833), there have been no dramatic changes in response rates in a variety of human tumors (49). Cancers such as acute myelocytic leukemia and myeloma, which commonly express P-gp at a low level at presentation and with increasing frequency following chemotherapy, may give higher response rates when a P-gp inhibitor is included in the chemotherapy regimen (49a). Solid tumors, such as renal cell cancer and colon cancer, do not respond significantly despite relatively high levels of expression of P-gp, suggesting that other mechanisms of drug resistance also contribute to the resistance of these solid tumors to many forms of chemotherapy (20). Recent knowledge about other ABC transporters such as MRP transporters and MXR that might contribute to drug resistance has caused clinicians to reconsider what the best inhibitors might be: a nonspecific inhibitor of ABC transporters that might have the broadest spectrum, but a higher likelihood of side effects, or a cocktail of specific inhibitors designed for each individual tumor. Also, there is a strong

need to be able to “image” activity of drug transporters in vivo using imaging agents, such as ^{99m}Tc -sestamibi, which are substrates for ABC transporters such as P-glycoprotein (50).

One consequence of enumerating the various transporters that handle anticancer drugs was the discovery that these transporters are also involved in pharmacokinetics of many different drugs in common clinical use. For example, P-gp is normally expressed at high levels in the mucosa of the GI tract, in biliary epithelial cells of the liver, in proximal tubule cells of the kidney, in the adrenal cortex, in capillary endothelial cells of the brain, testes and ovary, and in the placenta. These locations argue for a role of P-gp in excretion of drugs from intestine, liver, and kidney into stool, bile and urine; in blocking absorption of drugs from the GI tract; and as a barrier to transport into brain, testes, ovary, and the fetus (23).

The generation of a mouse lacking *mdr1a* and *mdr1b* by insertional mutagenesis demonstrated that loss of P-gp was not lethal to the animals, but they were very susceptible to toxic effects of many different drugs because of increased absorption and neurotoxicity (51). Studies with mice deficient in *mdr1a/mdr1b* and *mrp1* and cell lines derived from these triple knockout [*mdr1a/1b*(-/-), *mrp1*(-/-)] mice indicate that P-gp and MRP1 transporters contribute significantly to the development of resistance to paclitaxel (taxol), anthracyclines, and *Vinca* alkaloids (52). In addition, exposure of these triple knockout mice to therapeutic doses of vincristine resulted in severe damage to bone marrow and gastrointestinal mucosa, indicating that both P-gp and MRP1 are compensatory transporters for *Vinca* alkaloids in these tissues (53). These results suggest that P-gp and MRP1 are important determinants of the pharmacokinetics of many different drugs, and further, that inhibitors of P-gp and possibly MRP1 can be used to enhance uptake of these drugs that are given orally and perhaps to influence their penetration into the central nervous system. Evidence that P-gp serves a normal role in the physiological transport of opioid compounds out of the central nervous system into the bloodstream has also recently been reported (54). Recent data also suggest that a noncoding polymorphism in the P-gp gene is closely linked to levels of expression of P-gp in the GI tract, which results in alterations in absorption of commonly used drugs such as digoxin (55). It is assumed that pharmacogenomic analysis of other ABC transporters, such as the MRP family, will reveal similarly important roles in handling many different drugs.

The knowledge that expression of a drug-resistance gene, such as *MDR1*, can result in clinically significant drug resistance, has led to the idea that drug-resistance genes can be used as selectable markers in gene therapy (56). One of the barriers to successful gene therapy is the inefficiency of transfer of therapeutic genes into target cells. Use of cancer drug-resistance genes as linked markers to allow selection of cells to which therapeutic genes have been transferred has been suggested as a means to improve efficiency of gene therapy. Many different vectors have been developed using *MDR1* and other drug-resistance genes for gene therapy (57). Clinically, attempts have been made to introduce P-gp into bone marrow cells to protect them from the cytotoxic effects of anticancer drugs. In the mouse

this works reasonably well because efficiency of transfer of genes into mouse bone marrow stem cells is relatively high (58). In the human transfer efficiencies are low. Whereas expression of P-gp might have a small selective advantage after taxol treatment of patients undergoing bone marrow transplants in association with breast cancer treatment, the effect is not dramatic or therapeutic (59). In addition, some studies in the mouse suggest that high levels of P-gp in hematopoietic cells may be associated under some circumstances with a myeloproliferative disorder, and more data are needed before additional clinical trials can be attempted (60).

CONCLUSIONS

A great deal is now known about mechanisms of drug resistance in cancer cells. Despite the development of new targeted anticancer therapies, mechanisms that have evolved in mammals to protect cells against cytotoxic compounds in the environment will continue to act as obstacles to successful treatment of cancer. Additional knowledge about these mechanisms of cancer drug resistance may help to design strategies to circumvent resistance and new drugs that are less susceptible to known resistance mechanisms. Some of the knowledge about drug resistance has revealed new mechanisms relevant to normal handling of drugs by the body, and this information will be important in improving drug delivery and distribution in patients.

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