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Nanomedicinal strategies to treat multidrug-resistant tumors: current progress

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Abstract

Multidrug resistance (MDR) is a major impediment to the success of cancer chemotherapy. P-glycoprotein is an important and the best-known membrane transporter involved in MDR. Several strategies have been used to address MDR, especially P-glycoprotein-mediated drug resistance in tumors. However, clinical success has been limited, largely due to issues regarding lack of efficacy and/or safety. Nanoparticles have shown the ability to target tumors based on their unique physical and biological properties. To date, nanoparticles have been investigated primarily to address P-glycoprotein and the observed improved anticancer efficacy suggests that nanomedicinal strategies provide a new opportunity to overcome MDR. This article focuses on nanotechnology-based formulations and current nanomedicine approaches to address MDR in tumors and discusses the proposed mechanisms of action.

Keywords

Brij; drug delivery; efflux transporters; lipid formulation; nanoparticle; P-glycoprotein; Pluronic; vitamin E TPGS

Cancer is a leading cause of death worldwide, accounting for 7.9 million deaths in 2007 [201]. Over 85% of human cancers are solid tumors. Surgery and radiation are the initial treatments for most cancers. Chemotherapy with cytotoxic agents is used as an adjuvant therapy or a single-agent therapy especially for the management of recurrent disease. There are a number of chemotherapeutic agents with established first-line activity against cancer, with the anthracyclines [1] and taxanes [2] generally considered the most active. The relative benefits and toxicities of individual anticancer agents or combinations must be considered, as well as the treatment history and clinical status of the patient. Toxicity is one

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of the most critical issues in chemotherapy since most anticancer agents lack selective efficacy in cancer tissue. Another problem with conventional chemotherapy pertains to the challenge of delivery. The effectiveness of cancer chemotherapy in solid tumors depends on adequate delivery of therapeutic agents to tumor cells. The biological properties of the solid tumor, which limit the penetration of drugs into neoplastic cells distant from tumor vessels, include abnormal and heterogeneous tumor vasculature, interstitium, interstitial fluid pressure (IFP) and cell density. However, even if anticancer drugs are located in the tumor interstitium, they can have limited efficacy because cancer cells are able to develop mechanisms of resistance. Drug resistance has emerged as a major obstacle limiting the therapeutic efficacy of chemotherapeutic agents. These mechanisms allow tumors to evade chemotherapy. For example, multidrug resistance (MDR), a term to describe the broad-spectrum resistance to chemotherapy in human cancer, is one of the most important problems in chemotherapy. MDR is the phenomenon in which exposure of tumor cells to a single cytotoxic agent accounts for cross-resistance to other structurally unrelated classes of cytotoxic agents. ATP-binding cassette (ABC) transporters are transmembrane proteins that utilize the energy of ATP hydrolysis to shuttle various substrates across the cell membrane. Normally, ABC transporters function as pumps that extrude toxins and drugs out of the cell. To date, there are approximately 49 known transporters in the ABC family that are classified into seven different families (ABC A through ABC G) [3,4]. However, not all have been shown to have a role in MDR. Table 1 provides a summary of the most pertinent MDR-related ABC transporters and their most studied drug substrates [5]. P-glycoprotein (P-gp) is one of the most well-described ABC transporters and is overexpressed in the plasma membrane of MDR tumor cells. P-gp is capable of effluxing various anticancer drugs, such as doxorubicin and paclitaxel, out of the cells. P-gp inhibitors (e.g., verapamil) have been developed to overcome P-gp-mediated MDR. However, some P-gp inhibitors do not have good selectivity and also block normal cell function of P-gp, for example, in the intestines or at the blood–brain barrier (BBB), and therefore increase toxicity. A refinement of this concept is the incorporation of both the therapeutic drug and the P-gp inhibiting agent into the same drug carriers (e.g., nanoparticles) for simultaneous delivery into the cell.

Nanoparticles have been developed to enhance the intracellular concentration of drugs in cancer cells while avoiding toxicity in normal cells using both passive and active targeting. The nanosize particles can pass through leaky and hyperpermeable tumor vasculature and accumulate in the tumor vicinity utilizing the enhanced permeability and retention (EPR) effect. Moreover, vascular permeability-enhancing factors, such as bradykimin, nitric oxide and VEGF, facilitate extravasation of macromolecular drugs within tumor tissues, as well as surrounding normal tissues. Furthermore, the tumor interstitium is also characterized by the absence of an anatomically well-defined functioning lymphatic network. Therefore, the clearance of nanoparticles via lymphatics is generally seriously compromised in neoplastic tissues, so that an additional retention of nanoparticles in the tumor interstitium has been observed. This particular concept known as the EPR effect results in intratumoral drug accumulation, which is even higher than that observed in plasma and other tissues. In addition to passive targeting by the EPR effect, active targeting may also be pursued by targeting nanoparticles with a tumor cell-specific ligand. More importantly, it has been suggested that nanoparticles may be able to circumvent P-gp-mediated resistance. The mechanisms of carriers to overcome P-gp have been proposed based on various drug delivery systems, including *N*-(2-hydroxypropyl)methacrylamide (HPMA) drug conjugates, Pluronic® micelles, hybrid lipid nanoparticles, lipid-based nanocapsules and nanoparticles, liposomes and cyanoacrylate-type nanoparticles. Reported mechanisms included enhancement of cellular uptake of drug via endocytosis and ion-pair formation, ATP depletion, influence of function and expression of P-gp, and change of P-gp downstream signaling pathways.

Barriers in chemotherapy for the treatment of solid tumors

The development of therapeutic drugs against cancer has been focused on finding innovative approaches to selectively deliver anticancer drugs to solid tumors, while minimizing injury to healthy tissues. However, the efforts are confronted by physiological barriers at the tumor level and drug resistance at the cellular level.

Physiological characteristics of solid tumors related to drug delivery include: abnormal blood vessel architecture and function; interstitial fluid pressure, also known as interstitial hypertension; and lack of lymphatics [6,7]. Unlike normal microvessels, tumor vessels resulting from angiogenesis are dilated, tortuous and heterogeneous in their spatial distribution [8–10]. The flow of blood through tumor vessels varies according to tumor types and microenvironment, so that the penetration of drugs in solid tumors is difficult. The disorganized structure of solid tumors causes poor drug delivery to the central region in large tumors. Consequently, the microenvironment in solid tumors may also contribute to drug resistance by limiting drug penetration, thereby exposing the tumor to lower than efficacious concentration [11]. Nonetheless, large interendothelial junctions, increased numbers of fenestrations and abnormal basement membranes are often found in tumor vessels. The 'leakiness' of tumor vessels generates gap openings that are significantly larger than those observed in normal tissues [11–14]. This vascular permeability in solid tumors leads one to develop tumor-targeting carriers that are small enough to enter through tumor vascular openings, without passing through those in normal tissues. The abnormal structure and function of blood and lymphatic vessels in solid tumors cause IFP elevation whereas IFP in normal tissues is approximately 0 mm/Hg. An increase in tumor IFP reduces the transcapillary pressure gradient from the vascular compartment to interstitial space of tumors [15]. Furthermore, uniform elevation of IFP results in a negligible flow inside tumors [16]. Thus, the uniformly elevated IFP impedes the delivery of therapeutic drugs across both the blood vessel wall and interstitium in solid tumors. Studies have demonstrated that there are no functional lymphatic vessels inside solid tumors [17–19]. Functional lymphatic vessels are present in the tumor margin and the peritumoral tissue to mediate tumor growth and metastases. The lack of lymphatics in tumors reduces the effective outflow of interstitial fluid, partially contributing to the increase of tumor IFP. However, the positive aspect of the absence of lymphatics for drug delivery is that drugs can not be drained out of tumors via the lymphatic system once they locate inside tumors. Moreover, since a blood supply is crucial for growth, tumor cells have the ability to recruit new blood vessels in various ways through a process termed angiogenesis. The fact that the survival of a tumor critically depends on its blood supply provides a common opportunity for the destruction of solid tumors if drug concentrations in the bloodstream remain high for an appropriate period.

Therapeutic drugs will face another major barrier – drug resistance – after they penetrate in tumors. MDR remains the main cause of the failure of the use of conventional chemotherapy to treat common solid tumors [20]. Frequently, resistance is intrinsic to the cancer, but as therapy becomes more and more effective, acquired resistance has also become common. A variety of mechanisms contribute to acquired drug resistance to a broad range of anticancer drugs, including: expression of one or more energy-dependent transporters that bind to and efflux anticancer drugs from cells, leading to insensitivity to drug-induced apoptosis [21]; altered targets; and induction of drug-detoxifying mechanisms [22]. A summary of the reported mechanisms of drug resistance is presented in Figure 1. The initial MDR was reported in the late 1960s and early 1970s. Drug-resistant mammalian cell lines were then established for studying this phenomenon. A common feature of these drug-resistant cell lines was the overexpression of P-gp when compared with the drug-sensitive parent cell lines [23,24]. So far, overexpression of drug transporters is the most common reason for MDR. P-gp is an important and best-known membrane transporter involved in MDR. In

humans, closely related genes, *MDR1* and *MDR2* or *MDR3,* encode highly homologous P-gp. However, only the *MDR1* gene has been linked to the MDR. The *MDR1* gene is highly expressed in many clinically resistant tumors. In some cases its expression at diagnosis has been proven to be an adverse prognostic factor. In other cases, *MDR1* appeared after relapse from remission, suggesting that P-gp was a survival mechanism in a subclone of cancer cells that eventually reoccured [25–30]. Studies in leukemias, myelomas and some childhood cancers demonstrated that P-gp expression correlated with poor response to chemotherapy. Moreover, the early clinical trials testing P-gp modulation in acute leukemia in the presence of P-gp modulators resulted in clinical relapse and reduction of P-gp expression [31,32]. These clinical evidences demonstrated that P-gp plays a significant role in clinical drug resistance.

P-glycoprotein is an ATP-dependent transmembrane transporter that can transport a broad range of structurally unrelated compounds out of the cells. The human P-gp is a 170-kDa protein containing 1280 amino acids. The topology of P-gp was proposed to contain 12 transmembrane domains with six extracellular loops and two hydrophilic regions (ATP-binding domains), containing nucleotide-binding domains that are characteristics of the ABC family of transporters (Figure 2) [33–36]. P-gp is expressed at significant levels in human normal tissues, such as the biliary canaliculi of the liver, the proximal tubules of the kidneys, the small intestine, the colon and the adrenal cortex [37,38]. The activity of P-gp in normal tissues suggested an important role by transepithelial transport to prevent cytotoxic compounds in the environment and diet from entering the body, although the physiological role of P-gp remains a subject of speculation. Studies in P-gp-knockout mice demonstrated that P-gp had no exclusive and essential physiological function on fertility. The mice grew and developed into adulthood normally. However, these mice were very sensitive to MDR-related anticancer drugs. Moreover, more of these drugs accumulated in the CNS of the mice, indicating a major role of P-gp at the BBB [39–41].

P-gp can remove many different drugs from cells to decrease intracellular accumulation of anticancer drugs. Drugs may be pumped out by P-gp as they enter the plasma membrane, or even as they are inside the cells. These drugs include many of the commonly used natural product anticancer drugs, such as doxorubicin, vinblastine, etoposide and paclitaxel, as well as many commonly used pharmaceuticals, ranging from antiarrhythmics and antihistamines to cholesterol-lowering statins and HIV protease inhibitors [5,42]. Although the detailed mechanism of drug efflux by P-gp is unknown, the predicted transport cycle of P-gp involves the change of structure of ATP-binding domains after binding of drug substrates, stimulation of ATP hydrolysis, and rearrangement of P-gp shape caused by ATP binding and hydrolysis. The final step involving the conformational change of P-gp results in the release of the drug into the extracellular space. ATP hydrolysis is necessary for resetting of P-gp [43].

Seven strategies to overcome MDR

The strategies to overcome MDR are summarized in the following sections.

Modification of chemotherapy regimens

Noncross-resistant chemotherapeutic regimens utilize the largest number of active agents at the highest possible doses, assuming that mutations conferring drug resistance will not convey resistance to all of the agents in the regimen and, also, high-dose chemotherapy regimens could be given to cancer patients. This approach assumes that despite resistance to standard doses of anticancer drugs, a dose–response relationship still exists for these tumors and that high doses of chemotherapy might overcome this resistance.

Inactivation of MDR-associated genes by targeting specific mRNA for degradation

Antisense oligonucleotides and catalytic RNAs have successfully reduced P-gp, MRP and BCRP expression and sensitized drug-resistant cells [44–47]. The most recent development targeted to mRNA degradation is based on the RNA interference post-transcriptional gene silencing mechanism. Both siRNA (which has a transient effect) and shRNA (which has longer term effects) silence gene expression of P-gp, MRP and BCRP in MDR cancer cells [48–53]. To limit the exposure of normal cells to the inhibitor (siRNA) and the anticancer drug and maximize synergy, both P-gp-targeted siRNA and paclitaxel were coencapsulated into poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles. Biotin was also attached to the nanoparticles to target biotin receptors on cancer cells. *In vitro* and *in vivo* studies demonstrated that the biotin-functionalized nanoparticles encapsulating paclitaxel and siRNA partially overcame tumor drug resistance [54].

Use of monoclonal antibodies for P-gp

A monoclonal antibody to P-gp has been shown to inhibit tumor growth in an athymic nude mouse model [55,56]. However, there are some issues with the antibody approach; for example, MRK-16, a specific monoclonal antibody for P-gp, may target MDR1-expressing cells in normal tissues. Thus, the potential of this approach to lead to unacceptable toxicities could be overlooked in certain mouse models.

Development of new anticancer drugs that are not substrates of P-gp

The modified drug analogs can affect the binding of analogs to P-gp, and consequently P-gp cannot recognize the analogs. Two taxane analogs, DJ-927 (Phase I) [57,58] and ortataxel (Phase II) [59,60], designed to overcome drug resistance, have been evaluated in clinical trials. Other novel taxane analogs, such as BMS-184476 (Phase I) [61,62] and RPR 109881A (Phase II) [63,64], were also claimed to have a broad spectrum of activity both in sensitive and resistant tumor cell lines.

Use of inhibitors of ABC transporters to reverse MDR

This is an important approach receiving a great deal of attention. The inhibitors of ABC transporters can be classified into two groups. Some inhibitors can transport themselves and then act as competitive antagonists. The others are not transported but affect transporter function. The mechanism of reversal of transporters is discussed later.

Use of nanotechnology-based formulations & nanomedicine approaches to overcome MDR

Recently, several different formulations encapsulating anticancer drugs that are P-gp substrates have been developed. Paclitaxel vitamin E emulsion (TOCOSOL), containing the P-gp inhibitor D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) as an excipient, was evaluated in a Phase II clinical trial for drug resistance. The results showed promising efficacy when TOCOSOL was administrated weekly in patients with refractory cancers [65]. Partial reversal of drug resistance was also observed when liposomal doxorubicin was given to cell cultures [66]. This topic is discussed in greater detail in the sections below.

Inhibition of MDR using peptides

Synthetic P-gp-derived peptides corresponding to fragments from the extracellular loops of the murine P-gp were coupled to polyethylene glycol (PEG) and inserted into liposomes. These peptides have been shown to reverse MDR. After immunization with these peptide-loaded liposomes and treatment with doxorubicin, an increase of 83% survival time was observed in mice inoculated with P388R cells. No auto-immune responses were detected in immunized mice. However, complete eradication of tumors did not occur. These results

indicate a potential new approach to break immune tolerance towards MDR1 protein and consequently modulate sensitivity of resistant tumors to chemotherapy [67–69].

The clinical success has been limited in the attempts to overcome MDR. A large number of a new generation of drug analogs have been developed as non-P-gp substrates. However, most taxane analogs have not demonstrated greater therapeutic indexes *in vivo*, or have shown toxicity to normal tissues, although they were designed and shown not to be P-gp substrates [62]. Another means to overcome MDR is to develop ABC transporter inhibitors. Studies on compounds, including the calcium channel blocker verapamil, to reverse vincristine resistance in murine leukemia cells opened the door for the development of inhibitors of P-gp to overcome P-gp-mediated drug resistance [70,71]. The therapeutic results from previous clinical trials using first- and second-generation inhibitors of ABC transporters have generally been negative or only modestly positive and have not fulfilled the promise of the preclinical data. There are several reasons for the failure of many of these inhibitors to show beneficial effects. One of the reasons for failure is that the inhibitors are weak and nonspecific. The requirement for the use of high inhibitor concentration (e.g., >10 µM verapamil) was not achievable in clinical trials. Additional toxicities of anticancer drugs due to the inhibition of transporters in normal tissues remain a concern for the more potent inhibitors. Another reason for failure is the observed programmed pharmacokinetic drug interaction resulting from coadministration of inhibitors and anticancer drugs. For example, a modest pharmacokinetic interaction between verapamil and doxorubicin in humans was observed [72]. In addition, some second-generation inhibitors of P-gp are substrates of cytochrome P-450. Competition of these P-gp inhibitors for cytochrome P-450-mediated oxidative reactions may lead to undesirable pharmacokinetic interactions [73]. Increased toxicity of the anticancer drugs may occur due to both pharmacokinetic effects and inhibition of a protective function of P-gp in normal tissues. To date, clinical trials with the third generation inhibitors are ongoing, including trials with compounds LY335979-targeted P-gp, GF120918-targeted P-gp and BCRP, R101933-targeted P-gp and XR9576-targeted P-gp and MRP1. However, toxicity issues are still associated with this generation. For example, XR9576 demonstrated high potency in both *in vitro* and *in vivo* studies and showed no effect on the pharmacokinetics of several anticancer drugs in a Phase I study. However, a Phase III study in non-small-cell lung cancer has been stopped due to increased toxicity [74]. Therefore, the contribution of these inhibitors to reverse clinical drug resistance needs to be defined in clinical trials.

Nano-based drug delivery systems for cancer

In the pharmaceutical field, the development of the first viable nanocarrier dates back approximately 40 years, when the first example of a liposome was described [75]. However, the most important scientific advancements on development of nanoscale vehicles with distinct physical and biochemical properties for drug delivery applications have only taken place within the last two decades. The most common examples of these nanoparticles include polymeric nanoparticles, dendrimers, nanoshells, liposomes, lipid-based nanoparticles, magnetic nanoparticles and virus nanoparticles. One of the most appealing properties of nanoparticles is their size, resulting in distinct features that are not available from individual molecules alone or equivalent materials at a larger scale. Nanoparticles have emerged as one approach to overcome both the lack of specificity of conventional drugs and delivery barriers in solid tumors [76–82].

Nanoparticles have the potential to improve the therapeutic index of currently available drugs by increasing drug efficacy, lowering drug toxicity and achieving steady state therapeutic levels of drugs over an extended period of time. Nanoparticles can also improve drug solubility and drug stability, allowing the development of potentially effective new

chemical entities that have been stalled during the preclinical or clinical development owing to suboptimal pharmacokinetic or biochemical properties. Finally, the flexible surface chemistry of nanoparticles also allows for possible conjugation of targeting ligands for active targeted delivery.

A schematic representation for different mechanisms by which nanoparticles enhance drug delivery to solid tumors is shown in Figure 3 [76]. The effectiveness of cancer therapy in solid tumors depends on adequate delivery of the therapeutic agent to tumor cells. Many anticancer drugs have a marginal selectivity for malignant cells because they target the reproductive apparatus in cells having high proliferation rates. However, anticancer drugs having this mechanism of action result in high toxicities against rapidly dividing normal cells, such as hair follicles, germ cells and hematopoietic cells. Other anticancer drugs are distributed nonspecifically in the body where they affect cancer and normal cells. Therefore, the side effects associated with chemotherapy limit the dose achievable to the tumor and also result in suboptimal treatment due to excessive toxicities. Thus, the delivery of drugs to solid tumors remains difficult owing to the physiological barriers, as described above.

The role of tumor vasculature as a potential target for solid tumors has been elucidated since the late 1970s. In terms of the EPR effect, the small physical dimensions of nanoparticles enable them to partially penetrate through biological and physiological barriers of tumors that are unique to tumors and normally impermeable for larger particles. In this manner, passive targeted delivery to tumors based on the EPR effect may be achieved due to the physicochemical properties of a carrier and the pathophysiological condition of a target. For such a passive targeting mechanism to take place, physiochemical parameters of nanoparticles, including particle size and surface properties, are crucial. The desirable nanoparticles should have a certain particle size along with long blood circulation. The pore cut-off size of extravasation through transvascular gaps in most experimental tumors is in the range of 380–780 nm depending on the tumor types, whereas the tight endothelial junctions of normal vessels typically are between 1 and 10 nm [11,83]. It was proposed that transvascular transport of the particles in the tumors resulted from interendothelial or transendothelial open junctions rather than by endothelial phagocytosis or vesicles. To achieve this extravasation in solid tumors, an average particle size of less than 300 nm was suggested to be a requirement [11]. It should be noted there has been some recent debate as to whether the EPR effect is an artifact of mouse xenograft models, and whether this same type of effect exists in human cancers or even in genetically engineered mouse models [84,85]. If, in fact, human tumors lack the inherent leakiness required for the accumulation of nanoparticles by the EPR effect, then this will have profound effects on future nanomedicinal strategies for targeting solid tumors. As a consequence, one may have to develop strategies to enhance the convection of nanoparticles from the vasculature across the endothelial membrane to gain access to the solid tumors. Alternatively, one may have to employ anticancer strategies targeting the endothelium adjacent to solid tumors that may lead to an anticancer effect (e.g., using nanoparticles to deliver potent antiangiogenic factors perhaps in combination with drug[s]).

Another important prerequisite for passive targeted delivery is that the plasma concentration of the drug must remain high and long; meaning that drug-coated nanoparticles must be sustained in the bloodstream long enough to reach or recognize solid tumors. Nanoparticles, unlike microspheres (>1 μm), are small enough to be safely dosed via intravenous administration. In the bloodstream, a major defense system of the body, the reticuloendothelial system (RES), which is also known as the mononuclear phagocytes system, rapidly removes nanoparticles from the blood and becomes a major obstacle to long circulation of nanoparticles. The first step of clearance of nanoparticles by RES is opsonization, in which nanoparticles are recognized as foreign particles and are coated with

opsonin proteins to make them more recognizable to phagocytic cells. Phagocytic cells mainly include Kupffer cells of the liver and fixed macrophages of the spleen. A large number of researchers have been motivated to develop 'stealth' nanoparticles to avoid the uptake of nanoparticles by the RES. It has been repeatedly demonstrated that the opsonization of hydrophobic particles occur more quickly than that of hydrophilic nanoparticles, owing to the enhanced adsorbability of blood serum proteins on hydrophobic surfaces [86,87]. Modification of the nanoparticle surface with chains of hydrophilic and flexible polymers can shield nanoparticles from the opsonins and, therefore, prevent elimination by the cells of the RES. The most commonly used polymers are PEG, poly(ethylene oxide) and poly(propylene oxide), or their combinations. Such polymers can be put on the nanoparticle surface by covalently grafting, entrapping and adsorption. However, there are no absolute rules or methods available to completely and effectively block the opsonization of nanoparticles although research in this area has been ongoing for over 30 years. Modification of nanoparticle surface is complicated and case dependent. The thickness of the layer is crucial but hard to control and will vary within different types of nanoparticles and coating strategies. However, it is noteworthy that the effective stealth properties provided by these barrier layers depend largely on the PEG molecular weight, surface chain density and conformation [88]. In addition to clearance of nanoparticles by phagocytosis, nanoparticles are also subjected to splenic filtration, a non-phagocytic uptake process. The width of interendothelial cell slits of the spleen is approximately 200–500 nm [89]. A study of the biodistribution of polystyrene nanospheres (60–250 nm), coated or uncoated with hydrophilic polymers, was carried out in rats. In this study, coating of nanospheres with hydrophilic polymers dramatically reduced uptake of particles by liver, regardless of particle sizes. However, coated nanospheres were sequestered by the spleen, apparently depending on the particle size. The size and deformability of nanoparticles play critical roles in their clearance by this mechanism in human spleens. For rigid nanoparticles, the size to achieve long circulation should not exceed 200 nm. Otherwise, nanoparticles should be deformable enough to pass splenic filtration [90,91].

The innovations in combinatorial chemistry and high throughput screening, focused on achieving potency and high activity of small molecule anticancer drugs, are leading to a shift of the chemical and physical properties of new chemical entities towards more lipophilic and poorly water-soluble molecules. As a result, approximately 40% of potential drug candidates never enter further development, such as a formulation development stage, due to their poor water solubility [92]. The advances of lipid-based nanoparticle systems for improved drug delivery offer a great potential for the administration of anticancer drugs. The main advantages of these systems includes low toxicity of carriers themselves, solubilization of poorly water-soluble drugs, high drug loading, and protection of drugs against chemical and biological degradation related to the administration route, prolonged circulation, targeting delivery and controlled release. In addition, more than one anticancer drug may be coencapsulated into colloidal carriers for combined cancer chemotherapy [93]. Lipid-based nanoparticles are generally comprised of biocompatible and biodegradable lipids and, therefore, may be less toxic than many types of polymeric nanoparticles prepared from synthetic polymers. Once the particle size is reduced to sub-micron levels, the surface area of the nanoparticles increases substantially, which the saturation solubility also increases with the reduction of particle size, leading to an increase in the dissolution rate. The encapsulation of drugs into the carriers provides protection of the drugs from the influence of physiological conditions. Furthermore, the carrier may have prolonged circulation; thus, the required therapeutic levels of the drugs in the blood for the extended time interval, and a better targeting effect, may be more readily achieved. In addition, by choosing the excipients and compositions of the nanoparticles, controlled release can be explored to further reduce acute toxicity. As described previously, nanoparticles may be passively targeted to solid tumors by the EPR effect or even actively targeted by placing the ligand on the surface of

nanoparticles. Lipid-based nanoparticles have become an increasingly important field of research for delivering anticancer drugs in terms of these potential benefits. The primary types of lipid-based nanoparticles investigated have been liposomes, micelles, nanoemulsions, nanocapsules and solid lipid nanoparticles. These, as well as some other important systems, will be discussed in the following sections. All are potential drug carriers administered by different routes, including oral [94,95], topical [96,97] and parenteral [98,99].

Drug delivery systems to overcome P-gp-mediated drug resistance & their possible mechanisms

As mentioned previously, MDR is a major impediment to the success of cancer chemotherapy. P-gp was the first multidrug transporter discovered, and remains the best characterized and most clinically relevant to date. As such the majority of clinical trials have focused on P-gp, as highlighted in Table 1. P-gp is the product of the *MDR1* gene and effluxes drugs without chemical modification. Approximately 50% of the anticancer drugs used clinically today are substrates of P-gp [100]. The emergence of drug resistance has made many of the chemotherapy drugs ineffective. Different strategies attempting to overcome P-gp-mediated drug resistance have been developed as previously described. Therefore, this section focuses on overcoming P-gp-mediated drug resistance using drug delivery systems. Unlike the potentially more serious effects of the active P-gp inhibitors, drug delivery systems may be inhibitors of P-gp with low pharmacological activity and reduced side effects. The formulations discussed here to overcome MDR are summarized in Table 2 and will be highlighted in the following sections.

P-gp inhibition by surfactant-based formulations

In 1972, a published study demonstrated that Tween 80 enhanced the cytotoxicity of actinomycin D and daunomycin in Chinese hamster resistant cells [101]. Since this report, a number of lipid and polymeric excipients present in pharmaceutical formulations have been reported to modulate the activity of P-gp. P-gp, an efflux pump located in the apical membranes of intestinal absorptive cells, can reduce the absorption of drugs and consequently decrease the oral bioavailability. Thus, the lipid formulation strategy for enhancing absorption of drugs that are P-gp substrates became an attractive topic and has been extensively reviewed for oral delivery [100,102–104]. Caco-2 and MDCK cells expressing P-gp are the most widely used cell models to study the oral absorption. Most of the surfactants inhibiting P-gp are nonionic. They can be divided into two classes according to the chemical structure [102]. The first class of surfactants exhibit a hydrophilic head group and a hydrophobic tail responsible for membrane anchoring, including triglycerides, Cremophor EL, Solutol HS-15, vitamin E TPGS, Tween 80 and Brij 35. The second class of surfactants, which lack a typical membrane anchor, includes PEG and polyethylene oxide (EO)–polypropylene oxide (PO) block copolymers (Pluronics or poloxamers). All surfactant inhibitors contain a unit that comprises hydrogen bond acceptor groups such as ester groups and polyoxyethylene sequences, which could form hydrogen bonds with the transmembrane sequences of P-gp, which are rich in hydrogen bond donor groups. Provided that the binding affinity of surfactants is higher than that of the drug, the surfactants may be used to inhibit P-gp and, therefore, enhance drug absorption. The inhibitory effects of surfactants on P-gp efflux are related to the structure of surfactants, such as the length of the hydrophilic chain and hydrophilic–lipophilic balance (HLB). TPGS 1000 has been reported to influence drug efflux well below its reported critical micelle concentration (CMC) of 0.02 wt% [105]. A structure–activity relationship study was carried out to understand the influence of PEG chain length on apical efflux transporters in Caco-2 cell monolayers [106]. TPGS analogs containing different PEG chain lengths (molecular weight from 200 to 6000 Da) were

synthesized. The results suggested that PEG chain length was essential to influence rhodamine 123 efflux *in vitro*. TPGS 1000 turned out to be one of the most potent analogs to inhibit P-gp efflux. The effect of HLB values of excipients on P-gp modulation was also evaluated for their effects on the uptake of epirubicin in Caco-2 cells [107]. Surfactants with enhanced efficacy in this study, including Tween 20, Tween 80, Brij 30 and Myrj 52, consisted of a polyethylene and intermediate hydrocarbon chain. The characteristics of the surfactants allow them to partition between lipid bilayers and P-gp domains. The optimal HLB value to enhance epirubicin uptake was in the range of 10 to 17. A related study on Pluronic block copolymers with varying length of EO and PO segments was performed in bovine brain microvessel endothelial cells [108]. The most efficacious block copolymers exhibited intermediate length of 30–60 PO blocks and a relatively hydrophobic structure (HLB <20; e.g., Pluronic P85 with 40 PO blocks and an HLB of 16). The common mechanisms of surfactants to inhibit P-gp may include binding competition of drugs with surfactants resulting from an interaction between surfactants and P-gp, and membrane fluidization leading to an indirect protein destabilization [108–111]. However, the latter mechanism may not be the case for some surfactants. For example, TPGS tends to make lipid bilayers more, rather than less, rigid [110].

Liposomes

Liposomes have been, and continue to be, the most intensively researched colloidal drug delivery systems even for more than four decades after their discovery. Liposomes are normally composed of phospholipids that spontaneously form multilamellar, concentric bilayer vesicles, with layers of aqueous media separating the lipid layers. The particle size of small unilamellar vesicles, which are comprised of a single, lipid outer layer with an aqueous core, is in the range 20–80 nm. The surface of the liposomes may be charged or uncharged based on the selection of different phospholipids. There are many methods to prepare liposomes, including precipitation [112]. Liposomes may be used to load both hydrophobic and hydrophilic drugs. Hydrophilic drugs reside in the aqueous core, whereas hydrophobic drugs tend to remain in the lipid layers. Hydrophobic drugs are added during the formation of liposomes. Hydrophilic drugs may also be loaded during formation, but for charged drugs the pH-gradient method may be used wherein a pH gradient between the internal and external aqueous domains drives the drug into the interior of the liposomes by partitioning through the membrane. Liposomes have poor loading capacity for hydrophobic drugs that cannot be dissolved in sufficient amounts in the phospholipid bilayer or sequestered in the liposome core. Furthermore, after intravenous administration, such drugs often rapidly partition from the bilayers into cells, or bind to serum proteins, preventing accumulation at the target site. Several liposomal drugs are now marketed including Ambisome® (amphotericin B), Doxil® (doxorubicin hydrochloride) and Visudyne® (verteporfin) to name a few.

Liposomal delivery systems have been shown to inhibit P-gp efflux [66,113–117]. The proposed mechanisms included bypassing P-gp through an endocytosis pathway [117] and direct interaction with P-gp. The interaction of liposomes with P-gp was proved by complete inhibition of photoaffinity labeling of P-gp by azidopine [114]. However, other studies demonstrated that liposomes had limited effectiveness in addressing P-gp-mediated resistance in laboratory *in vitro* models of cellular resistance and in clinical studies [118–121]. Liposome formulations containing both an anticancer drug and a P-gp inhibitor have been studied recently. The results showed that liposomal coencapsulated drugs had better responses in both *in vitro* and *in vivo* resistant models compared with a single drug [122–124]. Moreover, liposomal targeting delivery systems have been investigated to overcome P-gp-mediated drug resistance [123]. For example, doxorubicin and verapamil were coencapsulated into liposomes with 95 and 70% loading efficiency, respectively. To achieve

active targeting, human transferrin (Tf) was conjugated to the liposomes to target Tf receptors, which are overexpressed in leukemia cells. In resistant leukemia K562 cells (Tf receptor[+]), Tf-conjugated coloaded liposomes showed 5.2- and 2.8-times greater cytotoxicity than nontargeted coloaded liposomes and Tf-conjugated doxorubicin liposomes, respectively. It was concluded that TfR-targeted liposomes coloaded with doxorubicin and verapamil were effective in selective targeting and reversal of drug resistance in cells [123].

Polymer, lipid nancapsules & nanoparticles

Nanocapsules have a liquid core (generally an oil) surrounded by a polymeric membrane structured by polymers or a combination of hydrophilic/lipophilic surfactants. Vegetable oils and triglycerides with medium- and long-chain fatty acids are the common components for the lipid cores. The drugs are confined to the lipid core, which serves as a reservoir to allow a high drug loading for hydrophobic drugs and a slow release profile. Thus, nanocapsules are pharmaceutically attractive for water-insoluble drugs.

Solid lipid nanoparticles made from biodegradable or biocompatible solid lipids were developed in the beginning of the 1990s as an alternative colloidal carrier system for controlled drug delivery. Solid lipid nanoparticles are matrix systems in which the drug is physically and uniformly dispersed. The release of a drug incorporated in the lipid matrix occurs due to degradation of the particles by lipases present at the site of injection, leading to a prolonged release of drugs from the solid lipid nanoparticles [125]. A comprehensive review on solid lipid nanoparticles can be found in the literature [126].

A number of studies have investigated encapsulation of anticancer drugs into polymer nanoparticles and lipid nanoparticles (or nanocapsules) to overcome P-gp-mediated drug resistance [127–132]. Among them, polyalkylcyanoacrylate nanoparticles were the earliest ones investigated in resistant cell lines [130]. The results showed that nonbiodegradable polymethacrylate nanoparticles can be internalized by an endocytosis process and reverse P-gp-mediated drug resistance *in vitro* [133]. For *in vivo* studies, biodegradable doxorubicin-loaded polyisohexylcyanoacrylate (PIHCA) nanoparticles were developed. These PIHCA nanoparticles showed more cytotoxicity than free doxorubicin in doxorubicin-resistant C6 cells. Later on, more rapidly biodegradable PIBCA nanoparticles were formulated to load doxorubicin. Doxorubicin uptake from PIBCA nanoparticles was different than that from PIHCA nanoparticles, as doxorubicin-loaded PIBCA nanoparticles caused higher cellular doxorubicin uptake than free doxorubicin. In addition, it was demonstrated that PIBCA nanoparticles did not enter cells via an endocytosis pathway and efflux of doxorubicin in nanoparticles had a similar profile with free doxorubicin. Mechanistic studies found that nanoparticles could deliver a high concentration of doxorubicin close to or adhered onto the cell membrane, resulting in saturation of P-gp; the formation of an ion pair between cyanoacrylic acid (a nanoparticle degradation product) and doxorubicin could also mask the positive charge of doxorubicin and facilitate diffusion of doxorubicin across cell membranes [134,135]. However, *in vivo* studies using MDR tumors demonstrated that these nanoparticles were not efficacious *in vivo*, perhaps due to poor delivery to the tumors [136]. A new polymer–lipid hybrid nanoparticle (PLN) system was used to increase the cytotoxicity of doxorubicin in resistant cells [132]. Doxorubicin uptake and retention from doxorubicin-loaded nanoparticles were significantly enhanced compared with free doxorubicin. Blank PLNs did not improve doxorubicin uptake and retention in resistant MDA-MB-435/LCC6MDR1 cells. These results indicated that the PLNs did not influence P-gp activity by themselves. The results also revealed that phagocytosis was an important pathway for PLN to enter the cells. Based on this pathway, doxorubicin-loaded PLNs could bypass P-gp, leading to enhanced doxorubicin uptake in resistant cells [137]. Recently, sodium bis(2-ethylhexyl) sulfosuccinate (AOT)–alginate nanoparticles were evaluated for their potential to overcome P-gp-mediated drug resistance. Doxorubicin-loaded AOT–

alginate nanoparticles enhanced the cytotoxicity of doxorubicin in resistant NCI/ADR-RES cells. It was observed that: the uptake of rhodamine was significantly increased using rhodamine-entrapped nanoparticles in resistant cell; blank nanoparticles improved rhodamine accumulation in a dose-dependent manner in resistant cells; and the enhancement in rhodamine accumulation was not due to membrane permeabilization. However, the mechanism of AOT–alginate nanoparticles to overcome P-gp-mediated drug resistance has not been established [129]. As mentioned previously, the surfactant solutol HS-15, a mixture of free PEG 660 and PEG 660 hydroxystearate, could inhibit P-gp. Solutol HS-15-based lipid nanocapsules (LNC) containing paclitaxel or etoposide were studied for their potential to overcome MDR [138,139]. Paclitaxel-loaded LNCs were shown to significantly reduce cancer cell survival in comparison with Taxol in 9L cells and F98 cells. Solutol HS-15 on its own did not improve the effects of paclitaxel. Similarly, paclitaxel-loaded LNCs significantly reduced tumor mass *in vivo,* whereas Taxol did not have a significant effect in an MDR-expressing F98 subcutaneous glioma model. The mixture of solutol HS-15 and paclitaxel did not improve tumor responses in this *in vivo* model. This indicated the importance of the nanocarrier itself for the anticancer effect on MDR. The study also showed that LNC internalization was not mediated by clathrin-dependent endocytosis. It was assumed that LNC endocytosis involves one or both of the two known cholesterol-enriched membrane microdomains. The consecutive intracellular cholesterol movements would constitute the core of the LNC inhibitory effects on MDR. Thus, according to these mechanism studies, the investigators proposed that the inhibition of the MDR efflux pump by LNCs could result from the interaction of the released intracellular free Solutol HS-15 with MDR efflux pump and redistribution of intracellular cholesterol [138]. Recently, doxorubicin and paclitaxel-loaded lipid-based nanoparticles containing Brij 78 as a surfactant were used to overcome P-gp-mediated drug resistance. These drug-loaded nanoparticles showed six- to nine-fold lower IC_{50} values in P-gp overexpressing human cancer cells than those of free drugs [140]. Paclitaxel nanoparticles showed marked anticancer efficacy in a nude mouse HCT-15 xenograft model via intratumoral injection [141] and in a nude mouse NCI/ADR-RES xenograft model after intravenous injection [140] as compared to all control groups. A series of *in vitro* cell assays were used to understand the underlining mechanisms. Enhanced uptake and prolonged retention of doxorubicin were observed with nanoparticle-based formulations in P-gp-overexpressing cells. Calcein acetoxymethylester assays and ATP assays confirmed that Brij 78 and blank nanoparticles inhibited P-gp and transiently depleted ATP. The change in the mitochondrial potential and mitochondrial swelling were observed to be dominant in MDR cells, suggesting Brij 78 and nanoparticles influence the mitochondrial respiratory chain. It was concluded that nanoparticles may be used to target both drug and biological mechanisms to overcome MDR via P-gp inhibition and ATP depletion [140]. It is noteworthy that the drugs delivered into MDR cells by PLGA nanoparticles are susceptible to efflux by P-gp [142]. PLGA nanoparticles were taken up by cells via endocytosis, resulting in an increase of cellular concentration of the drug encapsulated into the nanoparticles. Following entry, nanoparticles were retained in the cytoplasm for a sustained period of time and slowly released the drug in the cellular cytoplasm. However, paclitaxel-loaded PLGA nanoparticles did not show significant cytotoxicity in resistant NCI/ADR-RES cells. It was proved that P-gp activity did not affect the uptake and retention of nanoparticles themselves. Thus, the inefficiency of paclitaxel-loaded PLGA nanoparticles could result from the active efflux of the drug in cytoplasm by P-gp. P-gp could not only extract the drug when the drug diffused into the cell through the lipid bilayer, but also pump out the drug present in the cytoplasm. Consequently, the efficiency of overcoming P-gp based on endocytosis of nanoparticles may be limited.

Polymer–drug conjugates

Poly(*N*-[2-hydroxypropyl]methacrylamide) (polyHPMA) and HPMA copolymers have been proposed by several groups as potential drug delivery systems. HPMA is a water-soluble, non-immunogenic synthetic polymer. HPMA copolymer–doxorubicin conjugates have shown the potential to overcome drug resistance [143–145]. A series of studies on HPMA–doxorubicin conjugates addressed multiple mechanisms of MDR in addition to P-gp-mediated drug resistance. After the HPMA–doxorubicin conjugate was internalized by an endocytosis pathway, the spacer between the polymer and the drug was cleaved by an enzymatic hydrolysis reaction in the lysosomal compartment of the cells, resulting in the release of the drug from the conjugate. Chronic exposure of sensitive A2780 cells to HPMA–doxorubicin conjugates did not induce MDR as measured by quantification of *MDR1* gene expression; inhibition of *MPR* gene expression and a decrease of resistance against Taxol was evident [146]. By contrast, repeated exposure to free doxorubicin led to an increased resistance to doxorubicin and Taxol, and upregulation of the *MDR1* gene. Further *in vitro* mechanistic studies on overcoming MDR revealed that HPMA–doxorubicin conjugates inhibited: drug detoxification systems by suppressing the expression of genes encoding glutathione and UDP; and cellular defensive mechanism by activating apoptosis signaling pathways and downregulating the expression of the bcl-2 protein family and mechanisms of DNA repair, replication and synthesis leading to more DNA damage [147,148]. These results indicated that underlying mechanisms triggered by macromolecular carriers can modulate the biological response of the cell at a molecular level, resulting in an overall increased cytotoxicity. The ability of HPMA–doxorubicin conjugates to overcome MDR *in vivo* was demonstrated in solid tumor mouse models of sensitive human ovarian carcinoma A2780 and resistant A2780/AD tumors [149]. HPMA–doxorubicin conjugates significantly decreased tumor size by 28-fold in sensitive tumors and 18-fold in resistant tumors, whereas free doxorubicin only reduced tumor size by 2.8-fold in sensitive tumors and had no effect in the resistant tumor model as compared with the control. The underlying mechanisms were also investigated for the *in vivo* study. An enhanced accumulation of HPMA–doxorubicin conjugates in the tumor was observed and attributed to the EPR effect. The permeability of blood vessels decreased concomitantly with the downregulation of vascular growth and permeability (*VEGF*) gene in polymer-treated tumors. The other proposed *in vitro* mechanisms, such as downregulation of the expression of genes responsible for the activity of efflux pumps, detoxification and apoptosis, were also demonstrated in the *in vivo* studies.

Pluronic micelles

Micelles are the most basic colloidal drug delivery systems and are formed spontaneously in nature. In the body, colloidal micellar species comprising endogenous surfactants and lipid digestion products (i.e., bile salt mixtures) facilitate the absorption of highly insoluble fatty acids and fat soluble vitamins. Micelles have a particle size normally within a 5–100 nm range, are thermodynamically stable and form spontaneously by association of amphiphilic molecules, such as surfactants, under defined concentrations and temperatures. The concentration of a monomeric amphiphile at which micelles appear is termed the critical micelle concentration (CMC). The formation of micelles is driven by the decrease of free energy in the system owing to the removal of hydrophobic fragments from the aqueous environment and the re-establishment of the hydrogen bond network in water. Hydrophobic fragments of the amphiphilic molecules form the core of a micelle, while hydrophilic moieties form the shell of the micelle. When used as drug carriers in aqueous media, micelles solubilize molecules of poorly soluble nonpolar drugs within the micelle core, while polar drugs could be adsorbed on the micelle surface, and substances with intermediate polarity distribute along surfactant molecules in intermediate positions. One limitation of micellar systems is the relatively low hydrophobic volume of the interior of

micelles, leading to limited drug loading. Another limitation of conventional micellar systems is the danger of drug precipitation upon the dilution of the solubilized drug with physiological fluids after parenteral administration. The dilution of the formulation by physiological fluids may cause the disassociation of the micelles as the concentration of the surfactants used to solubilize the drugs may be lower than their CMC.

More recently, polymer micelles prepared from amphiphilic copolymers for solubilization of poorly soluble drugs as an alternative to lipid-based surfactant systems have attracted much attention. Polymer micelles offer a more versatile structure, biodegradability and lower CMC, which may lead to better *in vivo* stability and more conjugation chemistries for linking ligands to the surface of the colloidal system. Polymer micelles are self-assembled from block copolymers comprising a hydrophobic block (e.g., polylactic acid) with a hydrophilic block. As a result of a common progression of development of 'stealth' systems for intravenous administration, PEGylation approaches were used to form stealth micelles to enhance circulation time. Furthermore, the PEG corona can act as a diffusion barrier for hydrophobic drugs to reduce the burst release characteristic of drug-loaded micelles [150]. Thus, the hydrophilic block on the copolymer typically contains PEG segments with a molecular weight from 1 to 15 kDa. Similar to micelles formed with conventional surfactants, polymeric micelles comprise the core of the hydrophobic blocks stabilized by the corona of hydrophilic chains in water. However, compared with the conventional micelles, polymeric micelles are more stable upon the relatively high dilution conditions experienced *in vivo*. For example, some amphiphilic copolymers have CMC values as low as 10^{-6} M [151].

Pluronics are inert block copolymers comprising of poly(EO) (hydrophilic) and poly(PO) (hydrophobic). Pluronics are different from HPMA copolymers due to their amphiphilic nature. Their surfactant properties allow them to self-assemble into micelles with a hydrophobic PO inner core and a hydrophilic EO outer shell. However, similar to HPMA copolymers, Pluronic block copolymer micelles have also been shown to overcome drug resistance. Extensive studies with Pluronic block copolymer micelles have been reviewed [152–154]. SP1049C-containing doxorubicin in the mixed micelles of Pluronic L61 and F127 is in clinical trials to treat metastatic adenocarcinoma of the upper GI tract. In addition, SP1049C showed more efficient accumulation in tumors than free doxorubicin, while distribution of SP1049C in normal tissues was similar to that of doxorubicin [155]. Efficacy of SP1049C was confirmed in *in vivo* experiments in both sensitive and resistant tumor models, including P388 and P388/ADR murine leukemia, Sp2/0 and Sp2/0-Dnr murine myeloma, 3LL-M27 Lewis lung carcinoma, MCF-7 and MCF-7/ADR human breast carcinomas, and KBv human oral epidermoid carcinoma [153,155]. However, the toxicity of SP1049C was similar to that of free doxorubicin. This suggested that SP1049C did not improve the toxicity profile of free doxorubicin (e.g., cardiotoxicity), which was improved by liposomal doxorubicin. However, there were no additional side effects reported for SP1049C. Disintegration of micelles in biological fluids upon dilution to a concentration below its CMC is a common concern regarding using micelles for drug delivery. The biodistribution and pharmacokinetics of Pluronic P85 micelles suggested that the elimination of P85 was controlled by the renal elimination of P85 unimers and not by the rate of micelle disintegration [156]. However, micelle disintegration had been reported with other Pluronic micelles. To further address the potential of micelle disintegration, Pluronic L121 and Pluronic P-105 micelles were chemically modified to form a network or crosslink. Therefore, the CMC of the micelles was greatly reduced and the stability was enhanced, while their ability to inhibit P-gp function still remained [157,158].

The complex mechanisms associated with the effects of Pluronic block copolymers on MDR cells have been thoroughly studied. These mechanisms include altering membrane

microviscosity [159,160]. It was suggested that unimers (single block copolymer molecules) are responsible for biological modification as the effect of Pluronic copolymer occurred at concentrations below their CMC. The hydrophobic PO chains of Pluronic unimers insert into the hydrophilic regions of the membrane, resulting in alterations of the membrane structure, and decrease in its microviscosity (membrane fluidization); inhibiting drug efflux transporters, such as P-gp and MRPs, through inhibition of P-gp ATPase activity; and depleting intracellular ATP levels [159–161]. As these pumps are energy dependent, attenuation of these pumps was related to energy deprivation and abolishment of pump-associated ATPase activity. Thus, it can be surmized that Pluronic-mediated direct and indirect energy depletion leads to cessation of the operation of efflux pumps, and consequently sensitizes resistant cell lines to chemotherapeutic agents. A linear correlation between the extent of ATP depletion and chemosensitization elicited was established, further influencing cell apoptosis signaling. Doxorubicin-loaded Pluronic block copolymer P85 significantly enhanced the pro-apoptotic activity of the drug and prevented the activation of the antiapoptotic cellular defense [162]; decreasing glutathione (GSH) intracellular levels and glutathione-*S*-transferase (GST) activity, indicating the inhibition of the GSH–GST detoxification system [163]; and inhibited mitochondria respiratory chain and decreased oxygen consumption in MDR cells, accompanied by a decrease in mitochondria membrane potential, production of reactive oxygen species and release of cytochrome C. Eventually, this results in Pluronic-enhanced drug-induced apoptosis [164].

Conclusion & future perspective

The increasing importance of overcoming MDR in tumors has become emphasized in the last few decades. Great improvements in the application of nanotechnology as drug delivery systems have offered the possibility of more potential treatments for MDR. Commonly used pharmaceutical excipients have been explored for the ability to inhibit P-gp. This has, in turn, led to the development of several nanoparticle-based drug delivery systems that have incorporated these excipients to overcome MDR. Despite the fact that the mechanisms to overcome MDR using these nanoparticles are complicated and not fully understood, improved anticancer efficacy on MDR in tumors has been confirmed *in vitro* and *in vivo*. Moreover, some drug-loaded nanoparticles to treat MDR in tumors are now in human clinical trials. It is therefore anticipated that current development of nanoparticles may provide new strategies for the treatment of MDR.

However, many challenges remain for nanomedicine to overcome MDR. Drug-loaded nanoparticles still have the chance to distribute within normal tissues. Most of the current nanoparticles aimed at MDR utilize the EPR effect to pursue passive targeted delivery to solid tumors. Therefore, more efficacious targeting strategies for nanoparticles are still needed. Active targeted delivery could be achieved by attaching tumor specific antibodies or other ligands on the nanoparticle surface, or using external techniques (e.g., ultrasound, to enhance drug uptake at the tumor site). There also exists the need to continue to understand the biological mechanisms by which these nanomedicines overcome MDR. Although more research articles are published about the mechanisms, relatively few *in vivo* studies have been performed to date. More detailed studies on mechanisms would help direct the application of current delivery systems or lead to the discovery of alternative novel delivery systems. Furthermore, MDR itself is a complex phenomenon and contains different mechanisms. P-gp remains one of the best studied mechanisms, so most of the current formulation approaches have focused on P-gp-mediated drug resistance. Additional understanding of the mechanisms of how nanoparticles address the biological aspects of MDR may lead to novel nanoparticles that could effectively address MDR as well as other potential resistance mechanisms.

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Executive summary

Barriers in chemotherapy for the treatment of solid tumors

- Physiological characteristics of solid tumors present a barrier in chemotherapy as they result in poor drug delivery and therapeutic outcomes.
- Many cancer drugs will face multidrug resistance.

Strategies to overcome P-glycoprotein-mediated drug resistance

- Strategies include:
    - Modification of chemotherapy regimens
    - Inactivation of MDR-associated genes by targeting specific mRNA for degradation
    - Development of new anticancer drugs that are not substrates of P-glycoprotein (P-gp)
    - The use of inhibitors of P-gp to reverse P-gp-mediated drug resistance
    - The use nanotechnology-based formulations and nanomedicine approaches to overcome P-gp-mediated drug resistance
    - Inhibition of P-gp-mediated drug resistance using monoclonal antibodies or peptides

Nano-based drug delivery systems for cancer

- Nanoparticles may target solid tumors based on the enhanced permeability and retention effect to enhance tumor uptake and accumulation.
- Lipid-based nanoparticles offer a great potential to formulate poorly water soluble anticancer drugs.

Drug delivery systems to overcome P-gp-mediated drug resistance & their possible mechanisms

- Pharmaceutical excipients have shown the ability to inhibit P-gp and enhance drug uptake.
- Several drug delivery systems have been investigated to overcome MDR, including surfactant-based formulations, liposomes, polymer and lipid nanocapsules and nanoparticles, polymer–drug conjugates and micelles.

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Website
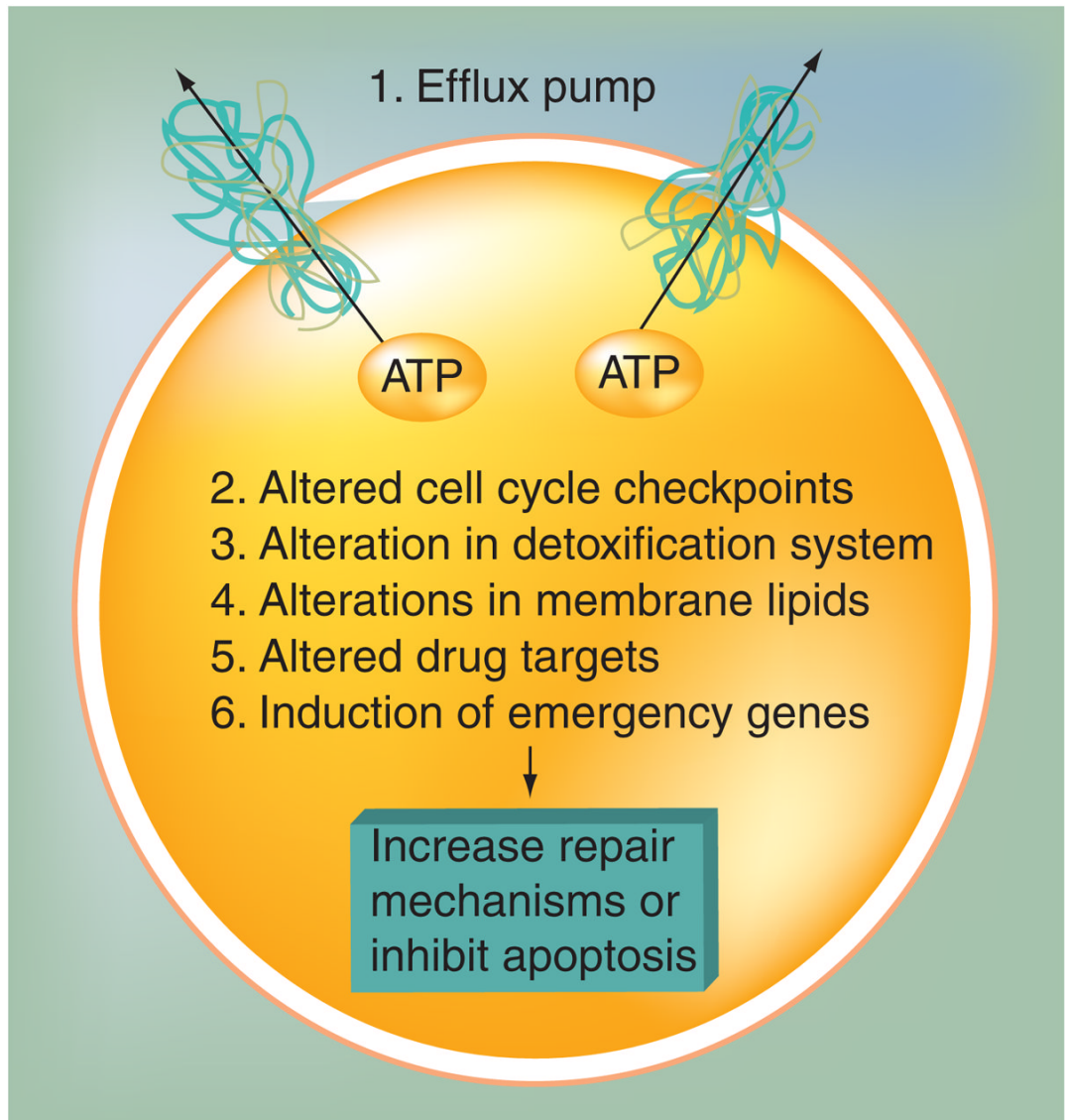
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Figure 1. Summary of the mechanisms in which cultured cancer cells have been shown to become resistant to cytotoxic anticancer drugs
The efflux pumps at the plasma membrane include P-glycoprotein, multidrug resistance protein family members and breast cancer resistance protein. Adapted from [42].
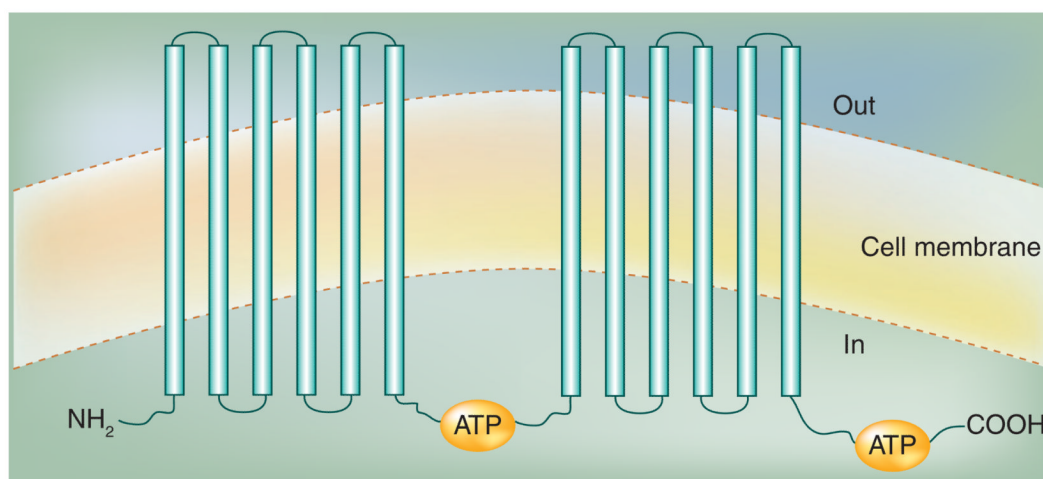
Figure 2. Topology model of P-glycoprotein
Each hydrophobic domain is followed by a hydrophilic domain (ATP-binding domain)
containing a nucleotide-binding site that is located at the cytoplasmic face of the membrane
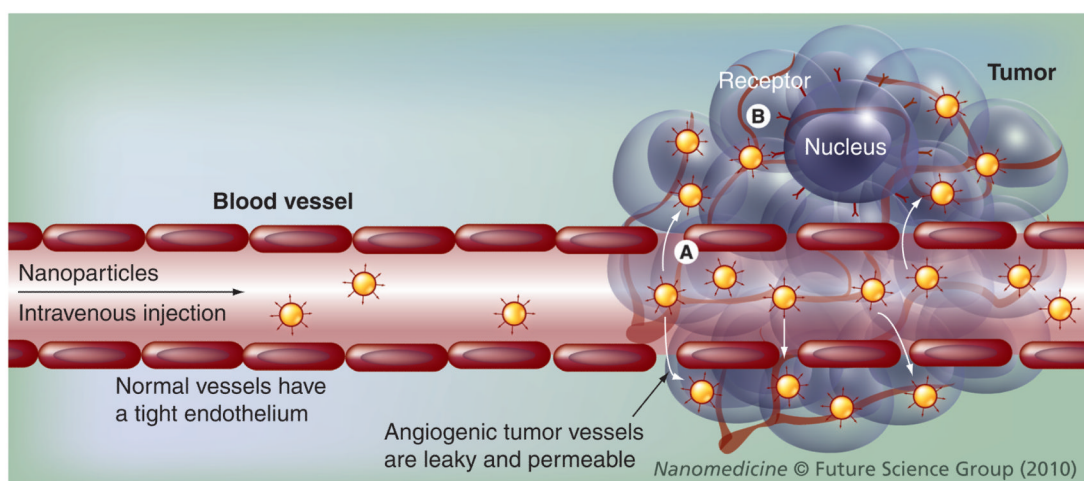and couples ATP hydrolysis.

Figure 3. Enhanced drug delivery to solid tumors using nanoparticles
(A) Passive targeted delivery. After intravenous injection, nanoparticles accumulate in tumors through leaky and permeable tumor vasculature and impaired lymphatic system (e.g., enhanced permeability and retention effect). **(B)** Active targeted delivery. Ligand-coated nanoparticles bind to a cancer cell receptor resulting in cell-specific recognition and improved drug delivery to solid tumors.

Table 1

Multidrug resistance-related ATP-binding cassette transporters and their substrates.

Subfamily	Genes	Established anticancer drugs	Published target for nanomedicine?	Ref.
ABC1	*ABC A2/ABC2/STGD*	Estramustine, mitoxantrone	No	
	ABCA3/ABC3	Daunorubicin, Ara-C, mitoxantrone and etoposide	No	
MDR/TAP	*ABC B1/MDR1/P-GP/PGY1*	Anthracyclines, vinca alkaloids, taxanes, etoposide, teniposide, imatinib, irinotecan, SN-38, bisantrene, colchicines, methotrexate, mitoxantrone, saquinivir, actinomycin D and others	Yes	[113–117,122–124,127–132,137–139,141,143–145,152–154]
	ABC B4/MDR3/PFIC3/PGY3	Daunorubicin, doxorubicin, vincristine, etoposide and mitoxantrone	No	
	ABC B5	Doxorubicin, camptothecin, 10-OH camptothecin and 5-FU	No	
	ABC B11/BSEP/PFIC2/SPGP	Paclitaxel	No	
CFTR/MRP	*ABC C1/MRP1*	Anthracyclines, vinca alkaloids, methotrexate, antifolate antineoplastic agents, etoposide, imatinib, irinotecan, SN-38, arsenite, colchicine, mitoxantrone, saquinivir and others	Yes	[53]
	ABC C2/MRP2/CMOAT	Vinca alkaloids, cisplatin, etoposide, doxorubicin, epirubicin, metotrexatetaxanes, irinotecan, SN-38, topotecan, arsenite, mitoxantrone and saquinivir	No	
	ABC C3/MRP3/CMOAT2	Etoposide, tenoposide and metotrexate	No	
	ABC C4/MRP4/MOATB	6-mercaptopurine, 6-thioguanine, irinotecan, SN-38, topotecan, AZT, metotrexate and PMEA	No	
	ABC C5/MRP5/MOATC	6-mercaptopurine, 6-thioguanine, irinotecan, 5-FU, cisplatin, AZT, metotrexate and PMEA	No	
	ABC C6/MRP6/MOATE/PXE	Etoposide, doxorubicin, daunorubicin, teniposide and cisplatin	No	
	ABC C10/MRP7	Taxanes and vinca-alkaloids	No	
	ABC C11/MRP8	6-mercaptopurine, 5-FU and PMEA	No	
WHITE	*ABC G2/BCRP/MXR/ABCP*	Mitoxantrone, camptothecin, anthracycline, etoposide, teniposide imatinib, flavopiridol, bisantrene, methotrexate, AZT and others	No	

5-FU: 5-fluorouracil; ABC: ATP-binding cassette; AZT: 3′-azido-2′,3′-deoxythymidine; CTFR: Cystic fibrosis transmembrane regulator; MDR: Multidrug resistance; MRP: Multidrug resistance protein; PMEA: 9-(2-phosphonylmethoxyethyl)adenine; TAP: Transporter associated with antigen presentation.

Adapted from [5].

Table 2

Formulations to overcome multidrug resistance and their proposed mechanisms.

Formulations	Proposed mechanisms	Status	Ref.
Liposomes	Endocytosis	*In vitro*	[117]
	Interaction of liposomes with P-gp	*In vitro*	[114]
	Coencapsulation of a P-gp inhibitor (verapamil)	*In vitro*	[123]
Polymethacrylate NPs	Endocytosis	*In vitro*	[133]
Polyisohexylcyanoacrylate NPs	No endocytosis	*In vitro*	[134–136]
	Saturation of P-gp by high concentration of the drug		
	Formation of an ion pair between NP degradation product and the drug	*In vivo*	
Polymer–lipid hybrid NPs	Phagocytosis	*In vitro*	[132]
AOT–alginate NPs	Not established	*In vitro*	[129]
Solutol HS-15-based lipid nanocapsules	Interaction of the released intracellular free solutol HS-15 with MDR efflux pump	*In vitro*	[138]
	Redistribution of intracellular cholesterol	*In vivo*	
Lipid-based nanoparticles containing Brij 78	Endocytosis	*In vitro*	[140,141]
	Inhibition of P-gp	*In vivo*	
	ATP depletion		
HPMA copolymer–doxorubicin conjugates	Endocytosis	*In vitro*	[143–149]
	Inhibition of drug detoxification systems	*In vivo*	
	Inhibition of cellular defensive mechanisms		
Pluronic® block copolymer micelles	Change in membrane microviscosity	*In vitro*	[152–161]
	Inhibition of drug efflux transporters	*In vivo*	
	ATP depletion	Phase II	
	Influence of cell apoptosis signaling		
	Inhibition of the GSH–GST detoxification system		
	Inhibition of mitochondria respiratory chain and decrease of oxygen consumption		

AOT: Sodium bis(2-ethylhexyl) sulfosuccinate; GSH: Glutathione; GST: Glutathione-*S*-transferase; HPMA: *N*-(2-hydroxypropyl)methacrylamide; MDR: Multidrug resistance; NP: Nanoparticle; P-gp: P-glycoprotein.