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Review

Overcoming drug efflux-based multidrug resistance in cancer with nanotechnology

Xue Xue and Xing-Jie Liang

Abstract

Multidrug resistance (MDR), which significantly decreases the efficacy of anticancer drugs and causes tumor recurrence, has been a major challenge in clinical cancer treatment with chemotherapeutic drugs for decades. Several mechanisms of overcoming drug resistance have been postulated. Well known P-glycoprotein (P-gp) and other drug efflux transporters are considered to be critical in pumping anticancer drugs out of cells and causing chemotherapy failure. Innovative theranostic (therapeutic and diagnostic) strategies with nanoparticles are rapidly evolving and are anticipated to offer opportunities to overcome these limits. In this review, we discuss the mechanisms of drug efflux-mediated resistance and the application of multiple nanoparticle-based platforms to overcome chemoresistance and improve therapeutic outcome.

Key words multidrug resistance, drug efflux transporter, cancer nanotechnology

According to the Global Cancer Report issued by the World Health Organization (WHO), there are over 10 million new cases of cancer each year and over 7.9 million annual deaths from the disease[1]. Serious toxicity is a critical problem for effective chemotherapy because most anticancer agents lack selective efficacy in tumors. Another vital issue is the development of tumor resistance to conventional chemotherapy. Drug resistance, which allows tumors to evade chemotherapeutic agents, has emerged as a major obstacle that limits the efficacy of chemotherapy. The formidable side effects of individual or combined anticancer agents as well as the treatment history and clinical status of the patient must be considered to circumvent the tumor resistance to chemotherapy in clinic. Tumors generally develop significant resistance to repeated treatment with one kind of anticancer agent and then often become resistant to similar or completely different drugs. This mechanism for tumor survival under chemotherapeutic treatment is known as multidrug resistance (MDR). MDR can be intrinsic or acquired through chemotherapeutic drug exposure, and multiple mechanisms are likely to contribute to clinical MDR. Historically, the most significant discovery about MDR was the identification of P-glycoprotein (P-gp)[2], which is overexpressed on the plasma membrane of cancer cells with MDR. Following P-gp, other transporters, such as multidrug resistance-associated protein 1 (MRP1)[3] and multixenobiotic resistance (MXR)[4], are also recognized to relate with drug efflux. In recent years, applications of nanotechnology have shown great promise, with several kinds of nanomedicine entering clinical studies. Although the feasibility and efficacy of reversing drug resistance has been confirmed *in vitro* and *in vivo*, the mechanisms by which to use nanotechnology to circumvent this phenotype have not been clarified or fully explored.

This article introduces nanotechnology-based formulations and possible nanomedical approaches to address MDR in tumors, with a specific focus on the use of nanotechnology in cancer to overcome drug efflux-mediated resistance.

Authors' Affiliation: CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Beijing 100190, P. R. China.

Corresponding Author: Xing-Jie Liang, CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Beijing 100190, P. R. China. Email: liangxj@nanoctr.cn.

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Possible Mechanisms of Drug Efflux-mediated Resistance in Cancer

The changes that drive antitumor drug resistance

include the following: increased activity of drug efflux pump, such as the ATP-binding cassette (ABC) superfamily; decreased drug influx; activation of DNA repair; metabolic modification or detoxification; and altered expression of apoptosis-associated protein Bcl-2 [5] and tumor suppressor protein p53 [6,7]. Of these mechanisms, overexpression of ABC transporters is the most frequent. ABC transport molecules are generally expressed on the plasma membrane and on the membranes of cellular vesicles, and they play vital physiologic functions and also affect the pharmacokinetic properties of chemotherapeutics in humans. ABC transporters are transmembrane proteins that use the energy of ATP hydrolysis to shuttle various substrates across the cell membrane. To date, there are 48 known transporters in the ABC family, which are classified into seven different subfamilies (ABC A through ABC G)[8-11] (Table 1). Thirteen ABC transporters contribute to tumor MDR, including P-gp (MDR1/ABCB1), multidrug resistance proteins (MRPs/ABCCs), and breast cancer resistance protein (BCRP/ABCG2), all of which are the most characterized ABC transporters[12]. The normal function of ABC transporters as pumps is to extrude toxins and foreign substances out of the cell. P-gp is the best-known membrane pump molecule of the ABC transporters involved in MDR. Human P-gp, a 170 kDa membrane-associated protein containing 1280 amino acids, is able to carry out an ATP-dependent conformational change that moves the intracellular substrates to the exterior of the cell[2]. It can transport a broad range of structurally related or unrelated compounds including anticancer drugs out of cells and thereby decrease intracellular accumulation of these compounds. Therefore, P-gp expression can physiologically prevent cytotoxic compounds by pump them out of cells to reduce their intracellular concentration. In patients with tumors, P-gp can efflux various anticancer drugs such as doxorubicin [13-15] and paclitaxel[16,17] out of cancer cells. Overexpression of P-gp is a common feature of most acquired MDR in solid tumors[18,19]. Other transporters such as MRP1, MRP2, and BCRP also contribute to drug distribution in the human body in cases where P-gp expression is not significantly altered after treatment.

Application of Nanoparticle Delivery Systems to Reverse Drug Efflux-mediated Resistance

As mentioned above, overexpression of ABC transporters is the broadly known tumor survival mechanism that limits the efficacy of chemotherapeutic agents in clinical cancer treatments. Currently, there are no traditional strategies without serious side effects to completely reverse chemotherapeutic resistance in tumors. Based on their unique physical and biological properties, cancer nanotechnologies developed in recent years offer an unprecedented opportunity for rational delivery of anticancer drugs to solid tumors [20,21]. The promise of nanotechnology lies in the ability to engineer customizable nanoscale constructs, which have more controllable surface for different modification, and to accommodate multiple types of payloads, such as cancer chemotherapeutics, chemosensitizers, or molecular imaging agents [22,23]. Nanoparticles have been developed to prevent, detect, and treat resistant cancer cells while minimizing serious toxicity in normal cells and improving drug solubility and stability. Nanostructure platforms derive their effectiveness from adequate delivery systems, including polymers, dendrimers, nanoshells, nanotubes, micelles, liposomes, lipid-based nanoparticles, magnetic nanoparticles, and virus nanoparticles. For rigid nanoparticles, the size to support long circulation *in vivo* may not exceed 200 nm, a size that is not achievable with individual molecules alone or with equivalent materials at a larger scale. Nanoscale drug delivery systems in the size range of 10–100 nm penetrate preferentially through the tumor vasculature via the so-called enhanced permeability and retention (EPR) effect [24-26]. Multifunctionalization of hydrophilic nanoparticles can provide a long circulating half-life and prolong the exposure time of chemotherapeutic drugs. These unique properties based on nanostructure effectively increase the intracellular accumulation of anticancer drugs by controllable and efficient release at targeted regions due to their size effect (Figure 1). Furthermore, properly designed nanoparticles with a targeting element can aim at specific targeting sites actively with the therapeutic payloads to overcome tumor MDR. One example is the use of folate acid as an active target. Nanoparticle with folate acid ligands can bind to folate receptors, which were found to be overexpressed on the surface of drug-resistant tumor cells, to achieve the specific accumulation in tumors. In addition, nanoplatforms offer opportunities to coencapsulate multiple therapeutic agents into a single functional carrier and allow imaging to be combined with drug treatment to monitor therapeutic effects in real time. Efforts to co-administrate drugs with ultrasound and thermosensitive therapy or photodynamic therapy improve the nanoparticle delivery systems for synergistic and comprehensive functions of cancer nanochemotherapy [27-30]. In summary, the advantages of nanoparticle-based drug delivery system include narrow size distribution, low carrier toxicity, enhanced drug solubilization via protecting drugs from efflux, prevention from drug metabolism or excretion before accumulating in tumors, increased drug loading, prolonged drug circulation, specific site targeting, and controlled drug

Table 1. Demographic and clinical characteristics of the studied population

Gene	Alias	Subfamily	Location	Gene size (bp)	dN/dS			Transcript	Protein	Main tissue expression	Function	Disease
					Dog	Mouse	Rat					
ABCA1	ABC1	ABC1	9q31.1	147154	0.078	0.048	0.072	1	1	Ubiquitous	Cholesterol efflux onto HDL drug resistance	Tangier's disease, familial hypoapo-proteinemia
ABCA2	ABC2	ABC1	9q34.3	21689		0.043	0.030	2	2	Brain	Drug resistance	
ABCA3	ABC3, ABCC	ABC1	16p13.3	53974	0.072	0.069	0.072	1	1	Lung	Phospholipid metabolism	Surfactant deficiency in newborns
ABCA4	ABCR	ABC1	1p21.3	128287	0.151	0.128	0.126	1	1	Photorecep-tors	N–retinylidene–PE efflux	Stargardt's disease/ fundus flavimaculatis, retinitis pigmentosa, cone-rod dystrpohy, age-related macular degeneration
ABCA5		ABC1	17q24.3	80499	0.132	0.091	0.087	2	2	Muscle, heart, testes		
ABCA6		ABC1	17q24.3	63169		0.356	0.282	1	1	Liver		
ABCA7		ABC1	19p13.3	10347		0.118	0.130	2	2	Spleen, thymus		
ABCA8		ABC1	17q24.3	88101	0.256	0.256	0.298	1	1	Ovary, heart, skeletal muscle, liver		
ABCA9		ABC1	17q24.3	86155	0.239	0.245	0.271	2	2	Heart		
ABCA10		ABC1	17q24.3	96808	NA	NA	NA	1	1	Muscle, heart		
ABCA12		ABC1	2q34	206885	0.113	0.075	0.101	2	2	Stomach	Glucosylceramide, other epidermal lipids	Lamellar ichthyosis type 2 (mild); Harlequin ichthyosis (severe)
ABCA13		ABC1	7p12.3	449249	0.397	0.221	0.218	1	1	Low in all tissues		
ABCB1	PGY1, MDR, P-gp	MDR	7p21.12	209617	0.180	0.16	0.145	1	1	Adrenal, kidney, brain, liver, intestine, testis, gland, uterus, ovary	Multidrug resistance	Ivermectin sensitivity, digoxin uptake
ABCB2	TAP1	MDR	6p21	8765	0.016	0.031	0.026	1	1	All cell	Peptide transport	Immune deficiency
ABCB3	TAP2	MDR	6p21	16912	0.225	0.204	0.207	2	2	All cell	Peptide transport	Immune deficiency
ABCB4	PGY3 or MDR3	MDR	7q21.12	75506	0.152	0.097	0.091	3	3	Liver	PC transport	Progressive familial intrahepatic cholestasis–3, intrahepatic cholestasis of pregnancy
ABCB5		MDR	7p21.1	108203	0.283	0.212	0.197	1	1	Ubiquitous		
ABCB6	MTABC3	MDR	2q35	9179	0.201	0.134	0.156	1	1	Mitochondria	Iron transport	Unknown
ABCB7	ABC7	MDR	Xq21–22	103026	0.215	0.11	0.059	1	1	Mitochondria	Fe/S cluster transport	X–linked sideroblastosis and anemia
ABCB8	MABC1	MDR	7q36.1	17116	0.135	0.137	0.150	1	1	Mitochondria		
ABCB9		MDR	12q24.31	46214	0.045	0.036	0.037	4	3	Liver		
ABCB10	MTABC2	MDR	1q42.13	42113	0.100	0.120	0.161	1	1	Mitochondria		

(*To be continued*)

Table 1. Demographic and clinical characteristics of the studied population (*continued*)

Gene	Alias	Subfamily	Location	Gene size (bp)	dN/dS			Transcript	Protein	Main tissue expression	Function	Disease
					Dog	Mouse	Rat					
ABCB11	SPGP or BSEP	MDR	2q24.3	108385	0.173	0.212		1	1	Liver, intestine	Bile salt transport	Progressive familial intrahepatic cholestasis-2
ABCC1	MRP1	CF/MRP	16p13.12	192840	0.064	0.060	0.062	7	7	Lung, testes, intestine, PBMC, kidney, brain	Drug resistance	
ABCC2	MRP2 or cMOAT	CF/MRP	10q24.2	69011	0.313	0.243	0.180	1	1	Liver, intestine, kidney	Organic anion efflux	Dubin-Johnson syndrome
ABCC3	MRP3	CF/MRP	17q21.33	56836	0.169	0.164	0.204	1	1	Lung, intestine, liver, kidney, placenta, pancreas, colon	Drug resistance	
ABCC4	MRP4	CF/MRP	13q32.1	281594	0.137	0.099	0.060	1	1	Prostate, lung, adrenal gland, ovary, testis	Nucleoside transport	
ABCC5	MRP5	CF/MRP	3q27.1	97956	0.069	0.073	0.053	1	1	Ubiquitous	Nucleoside transport	
ABCC6	MRP6	CF/MRP	16p13.12	73325	0.098	0.149	0.160	1	1	Kidney, liver	Nucleoside transport	Pseudoxanthoma elasticum
CFTR	ABCC7	CF/MRP	7q31.31	188699	0.172	0.172	0.238	1	1	Exocrine tissue	Chloride ion channel	Cystic fibrosis CBAVD, pancreatitis, bronchiectasis
ABCC8	SUR1	CF/MRP	11p15.1	84017	0.093	0.045	0.055	1	1	Pancreas	Sulfonylurea receptor	Familial persistent hyperinsulinemic hypoglycemia of infancy; AD type 2 diabetes
ABCC9	SUR2	CF/MRP	12p12.1	135631	0.021	0.039		3	3	Heart, muscle	Regulatory subunit of cardiac K(ATP) channel	Dilated cardiomyopathy with ventricular tachycardia
ABCC10	MRP7	CF/MRP	6p21.1	18675	0.170	0.195	0.171	1	1	Low in all tissues; a little higher in heart, skeletal, muscle, spleen, liver		
ABCC11	MRP8	CF/MRP	16q12.1	68267	0.304	NA	NA	2	3	Low in all tissues, a little higher in breast, and testis		
ABCC12	MRP9	CF/MRP	16q12.1	63798	0.200	0.184	0.166	1	1	Low in all tissues, a little higher in breast, testis, brain, ovary, skeletal muscle		
ABCC13												
ABCD1	ALDP	ALD	Xq28	19846	0.045	0.053	0.058	1	1	Peroxisomes	VLCFA transport regulation	Adrenoleukodystrophy

(*To be continued*)

Table 1. Demographic and clinical characteristics of the studied population (*continued*)

Gene	Alias	Subfamily	Location	Gene size (bp)	dN/dS			Transcript	Protein	Main tissue expression	Function	Disease
					Dog	Mouse	Rat					
ABCD2	ALDL1, ALDR	ALD	12q11	67424	0.085	0.066	0.073	1	1	Peroxisomes		
ABCD3	PXMP1, PMP70	ALD	1p22.1	100072	0.050	0.051	0.058	1	1	Peroxisomes		
ABCD4	PMP69, P70R	ALD	14q24.3	17540	0.188	0.112	0.110	1	1	Peroxisomes		
ABCE1	OABP, RNS41	OABP	4q31.31	30851	0.003	0.061	0.002	1	1	Ovary, testes, spleen	Oligoadenylate binding protein	
ABCF1	ABC50	GCN20	6p21.1	19920	0.022	0.057	0.055	1	1	Ubiquitous		
ABCF2		GCN20	7q36.1	19395	0.036	0.011	0.010	2	2	Ubiquitous		
ABCF3		GCN20	3q27.1	7908	0.027	0.030	0.035	1	1	Ubiquitous		
ABCG1	ABC8, White	White	21q22.3	97556	0.012	0.012	0.010	7	7	Ubiquitous	Cholesterol transport?	
ABCG2	ABCP, MXR, BCRP	White	4q22	66883	0.269	0.206	0.202	1	1	Placenta, intestine, brain	Toxin efflux, drug resistance	
ABCG4	White2	White	11q23	13626	0.026	0.064	0.027	1	1	Liver		
ABCG5	White3	White	2p21	26348	0.302	0.175	0.157	1	1	Liver, intestine	Sterol transport	Sitosterolemia
ABCG8		White	2p21	39503	0.152	0.126	0.206	1	1	Liver, intestine	Sterol transport	Sitosterolemia

ABC, ATP-binding cassette; HDL, high-density lipoprotein; PGY, P-glucoprotein; MDR, multidrug resistance; MXR, multixenobiotic resistance; MRP, multidrug resistance-associated protein; BCRP, breast cancer resistance protein; TAP, transporter ATP-binding cassette, ATP-binding cassette sub-family B; PC, phosphatidylcholine; MTABC, mitochondrial ATP-binding cassette; SPGP, sister of P-glycoprotein; BSEP, bile salt export pump; MRP, multiple drug resistance protein; PBMC, peripheral blood mononuclear cell; cMOAT, canalicular multispecific organic anion transporter 1; CF, cystic fibrosis; SUR, sulfonylurea receptor; ALD, adrenoleukodystrophy; ALDP, adrenoleukodystrophy protein; ALDR, adrenoleukodystrophy-related protein; PXMP, peroxisomal membrane protein; PMP, putative peroxisomal membrane protein; OABP, ATP-binding cassette, sub-family E; GCN20, ATP-binding cassette sub-family F; CBAVD, congenital bilateral absence of the vas deferens; VLCFA, very long chain fatty acid.

release. Cancer nanotechnology has the potential to reverse tumor MDR in a way that is not achievable with traditional strategies.

Cancer Nanomedicine for Overcoming Drug Efflux-mediated Resistance

An important prerequisite for reversing drug resistance in cancer is achieving a high concentration of the drug in the plasma, a high concentration and long retention time in MDR cancer cells to ensure effective intracellular accumulation. Considering different mechanisms of drug resistance in cancer, nanoparticles are always designed to inhibit or bypass efflux pumps on the membrane or to enhance endocytosis when recognizing MDR tumors. Over 50% of the anticancer drugs used in the clinic today are targeted by P-gp [31]. However, P-gp inhibitors do not have specific selectivity and also block the normal function of P-gp. Nanotechnology refines the concept of co-administering anticancer agents and P-gp inhibitors by combining them into a single drug carrier for simultaneous delivery into MDR tumor cells. In a recent review by Gottesman *et al.*[32], the strategies for circumventing P-gp-mediated MDR are eloquently categorized as follows: using P-gp inhibitors to block the efflux of cytotoxic agents; using drugs that are not substrates of P-gp; and exploiting the properties of MDR cells, such as receptor overexpression and collateral drug sensitivity. D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS 1000), which functions as an effective inhibitor of P-gp, turned out to be one of the prominent surfactants that enhancing the cytotoxicity of doxorubicin, vinblastine, paclitaxel, and colchicines in G185 cells comparable to that in the parental cells. Reversal of P-gp activity was due to the effect of TPGS 1000 on transport at concentrations even below its critical micelle point of 0.02 wt%. This excipient was evaluated in a phase II clinical trial for drug resistance [33]. Pluronic block copolymer (P85) is another important and promising example of a modifying agent for P-gp. Membrane fluidization by P85 treatment led to inhibition of the P-gp ATPase drug efflux system and to interference with metabolic processes. These results indicate that both energy depletion (via decreasing ATP pool necessary for P-gp function) and increased permeability and fluidization of a broad spectrum of drugs are critical factors contributing to the activity of the block copolymer for MDR reversion [34,35]. First-generation

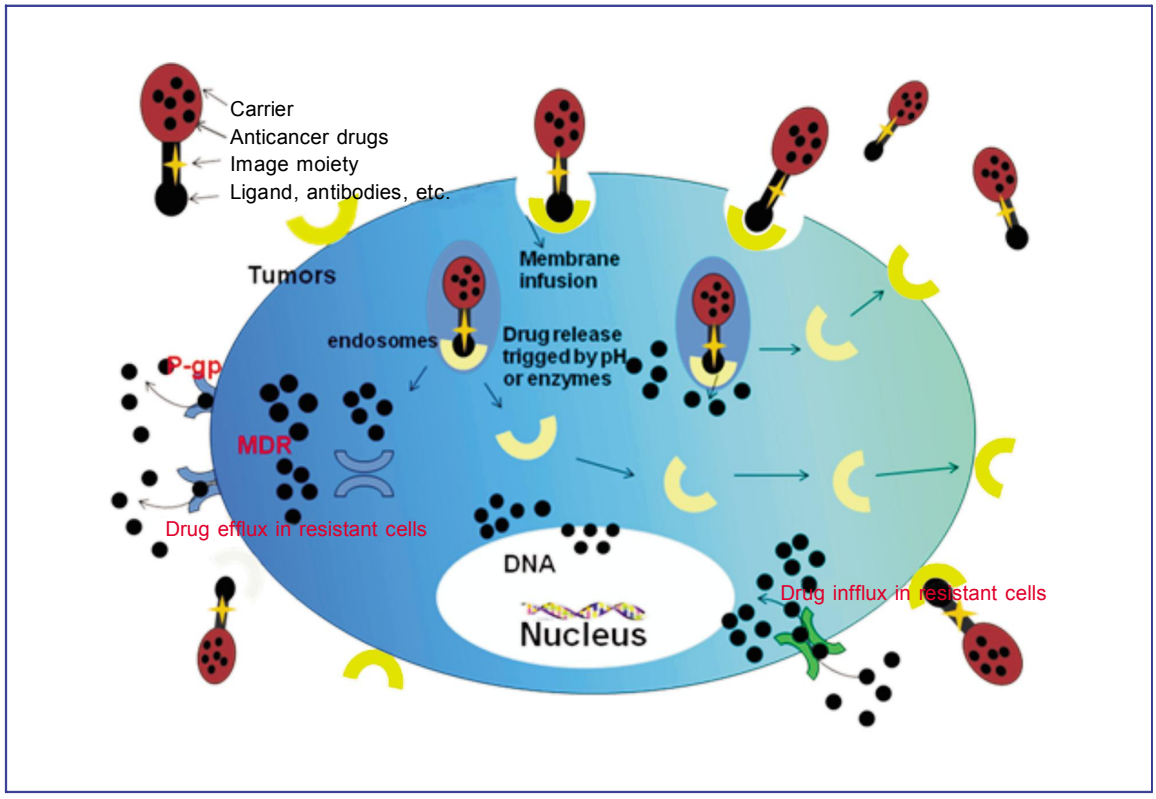

Figure 1. **Unique nanoplatforms effectively increase the intracellular accumulation of anticancer drugs by controllable and efficient release at targeted regions.** These functionalized nanoparticles can be modified with anticancer drugs, image moiety, specific ligands, antibodies, and so on, to fulfill chemotherapy through increasing drug accumulation.

agents (for example, the calcium channel blocker verapamil) are limited by unacceptable toxicity to normal tissues, whereas second-generation agents (for example, valspodar and biricodar) have better tolerability but are limited by nonspecificity. Third-generation inhibitors (for example, tariquidar XR9576, LY335979, GF120918, 9576, etc.) have high potency and specificity for P-gp. Tariquidar has shown marked effectiveness in early clinical trials. GF120918 has achieved adequate P-gp inhibition *in vivo* without significant side effects [36,37]. Taken together, the development of these findings suggests the necessity to consider to combine nanoparticle therapies with P-gp inhibitors.

Recent studies also demonstrated that nucleic acid (DNA, miRNA, siRNA, etc.)-based nanoparticles play a critical role in the modulation of drug resistance in tumors by effectively decreasing MDR1 expression *in vivo* [38-40]. The employed system, which uses siRNA to silence the expression of ABC transporters in combination with an appropriate anticancer drug, is a systemic administration strategy for MDR cells. Meng *et al.* [41] successfully achieved dual delivery of doxorubicin (Dox) and P-gp siRNA loaded in mesoporous silica nanoparticles (MSNP). P-gp gene knockdown by siRNA effectively increased the intracellular and intranuclear drug concentration. Similar results were also observed *in vitro* and *in vivo* using RGD peptide (arginine-glycine-aspartic acid)-modified liposomes containing P-gp siRNA or doxorubicin [42]. MacDiarmid *et al.* [43] provided a dual sequential treatment strategy for drug-resistant tumors with targeted micelles containing siRNA and a cytotoxic drug. First, the resistant tumors were treated with siRNA/shRNA-containing minicells targeted to tumors via bispecific antibodies (BsAb) for 48 h (for siRNA-containing minicells) and 144 h (for shRNA-containing minicells) to achieve substantial knockdown of MDR1. A second wave of treatment with siRNA/shRNA-containing minicells was followed with intravenous administration of BsAb-targeted minicells packaged with cytotoxic drugs to resistant tumor xenografts. Both the sequential and simultaneous approaches were effective and feasible and markedly decreased the doses of cytotoxic drug necessary for eliminating tumors. Injection of anti-MDR1 short hairpin RNA-encoding vectors into tumor cells with intravenous administration of doxorubicin completely reversed the MDR phenotype and inhibited tumor growth [44].

In addition, some nanomedicines that circumvent

MDR in cancer through other targets are being tested in clinical trials (Table 2). These include taxane analogs DJ-927 (phase I/II)[57-59] and ortataxel (phase II)[60,61], as well as BMS-184476 (phase II)[62-64] and RPR 109881A (phase I)[65-67], which were purported to have a broad spectrum of activity both in sensitive and resistant tumor cell lines. Nab-paclitaxel is a novel clinical entity incorporating paclitaxel into an albumin nanoparticle, leading to increased intratumoral concentration and showing with superior response rate, longer time to tumor progression, and prolonged survival as second-line therapy in patients with gynecologic cancers[68,69]. EGFR-targeted polymer-blend nanocarriers with a combination of paclitaxel and lonidamine were found to enhance the therapeutic index of both drugs by inhibiting the Warburg effect and promoting mitochondrial binding of pro-apoptotic Bcl-2 protein (via lonidamine), while hyperstabilizing microtubules (via paclitaxel)[70].

Future Perspectives

MDR is a major impediment to the success of cancer chemotherapy. P-gp is the best known membrane transporter involved in MDR in tumors. Several strategies have been used to address MDR, especially P-gp-mediated drug resistance in tumors. However, clinical success has been limited, largely due to lack of efficacy and/or significant toxicity. To overcome both the dose-limiting side effects of conventional chemotherapeutic agents and the therapeutic failure resulting from MDR, cancer nanotechnology has been developed and shown its ability to target tumors based on their unique physical and biological properties. To date, nanoparticles have been investigated primarily to address P-gp and have been shown to improve anticancer efficacy, indicating that nanomedical strategies might provide a new opportunity to overcome MDR[71]. The most predominant advantage of nanomedicine is to deliver and concentrate drugs at the plasma membrane where ABC transporters are located and saturated with extra drugs. In addition, functionalized nanoparticles themselves or their metabolites can also block the function of ABC transporters such as P-gp by direct or indirect interaction and inhibition. The flexibility of nanoparticles with regard to their size and shape increases their potential to enhance drug-loading capacity, stabilize drugs and regulate their release rates, and deliver drugs to targeted sites effectively and specifically. This article provides a glimpse into the nanotechnology-based strategies being developed to overcome drug resistance. To the best of

Table 2. Selected examples of nanoparticles used to overcome drug resistance in cancer treatment evaluated *in vivo* in the past three years

Nanotransporters	Main bioactive element	Main Mechanism	Reference
siRNA, minicells	Doxorubicin	P-gp inhibition	[43]
Dendrimer phthalocyanine-encapsulated polymeric micelle (DPc/m)-mediated PCI	Doxorubicin	P-gp inhibition	[45]
Poly(D,L-lactide-co-glycolide)	Paclitaxel, tariquidar	P-gp inhibition	[46]
Poly(D,L-lactide-co-glyco-lide) nanoparticles	Paclitaxel, *P-gp* targeted siRNA	P-gp inhibition	[47]
Albumin bound nanoparticles	Paclitaxel	Paclitaxel-induced NF-κB pathway that up-regulates VEGF-A	[48]
Aerosol-OT (AOT)	Doxorubicin	Prevents the accumulation of anticancer drugs	[49,50]
Folate receptor-targeting nanoparticle	Heparin-folate-paclitaxel (HFT) backbone with an additional paclitaxel	P-gp inhibition	[51]
PLGA nanoparticles	Vincristine sulfate, verapamil hydrochloride	Enhanced permeation and retention effect	[52]
Cationic liposome-polycation-DNA (LPD) and anionic liposome-polycation-DNA (LPD-II)	Doxorubicin and siRNA	Avoid P-gp efflux and increase Dox uptake	[53]
Polymer-blend nanoparticle	Ceramide	Modulation of the apoptotic threshold	[54]
Albumin bound nanoparticles	Rapamycin and perifosine	Suppression of the PI3K/Akt/mTOR pathway	[55]
Fe(3)O(4)-magnetic nanoparticle	Daunorubicin	Mdr-1 inhibition	[56]

P-gp, P-glycoprotein; aerosol-OT (AOT), bis-2-ethylhexoxyl sodium sulfosuccinate; PLGA, poly(lactic-co-glycolic acid); VEGF-A, vascular endothelial growth factor A; Dox, doxorubicin; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; mTOR, mammalian target of rapamycin; Mdr-1, multidrug resistant gene-1.

our knowledge, however, there are currently no nano-fomulations for drug delivery aimed at overcoming drug resistance that have been effective in clinical tests. Various nanocarriers for targeted delivery of anticancer drugs have already undergone *in vivo* testing in mouse models (Table 2) and clinical evaluation in humans. With better understanding of the physiologic properties of drug-resistant tumors and enhanced nanomaterial design, there will be more opportunities to develop multifunctional nanostructures for circumventing drug resistance. A safe and effective multifunctional nanosystem could provide a versatile platform to benefit patients with MDR tumors in the future.

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