

Chapter 15

Nanotherapeutics in Multidrug Resistance

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Abstract The development of resistance to a variety of chemotherapeutic agents, also known as multidrug resistance (MDR), is a main impediment to the success of cancer chemotherapy, which refers to many factors such as increased efflux, blocked apoptosis, decreased drug influx, and altered cell cycle regulation. Considerable efforts have been devoted to develop chemosensitizers to conquer drug resistance, while their safety and unwanted pharmacokinetic drug interaction hindered their clinical applicability. Nano-sized drug carriers have great superiority in overcoming drug resistance due to the improved therapeutic index of drugs, enhanced drug targeting in tumor sites, and success in escaping from recognition of ABC transporter-mediated drug efflux. This chapter summarizes the most recent developments in the field of nanotherapeutics toward overcoming drug resistance by drug-targeted delivery, increased intracellular availability, changed subcellular localization, and combination of drug delivery with the agents that regulate intracellular pH, energy delivery, and apoptotic threshold.

Introduction

Cancer is one of the major causes of death worldwide. Multidrug resistance (MDR), which is classically defined as a state of resilience against structurally and/or functionally unrelated drugs, is the main obstacle in cancer therapy. Generally, MDR can be divided into two types: intrinsic MDR and acquired MDR. Intrinsic MDR can be favored by the selection pressures in the tumor microenvironment, whereas acquired MDR can be induced by the traditional chemotherapy in common dose. Many factors can contribute to MDR, such as increased efflux, decreased drug influx, mutated cell cycle regulation, and blocked apoptosis.

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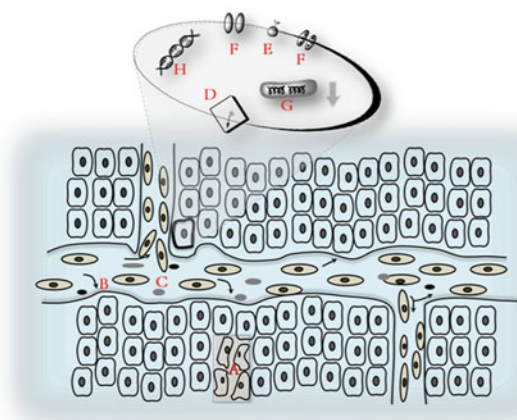
The intracellular concentration of drugs and therapeutic efficiency can be increased using nanovehicles, which can escape from the recognition of efflux pumps and thus be endocytosed by tumor cells. Moreover, drug resistance gene and protein inhibitors can also be loaded in vehicles to modulate cell apoptosis and intensify drug efficacy.

Tumor Microenvironment and Cancer-Initiating (Stem) Cells

The microenvironment of a tumor cell contributes to the development of MDR and determines cell response to chemotherapy. The microenvironmental selection pressures that contribute to the development of MDR also always make the cells hypersensitive to growth stimulation.

Meanwhile, complex phenotype transformations can occur in cancer cells under hypoxic conditions, which are necessary for cell survival under such conditions. This kind of survival is a cascade initiated by the translocation of the alpha subunit of hypoxia-inducible factor (HIF) from the cytoplasm to the nucleus. The translocation is followed by abnormal tumor vasculature, hypoxia, decreased pH, increased interstitial fluid pressure, and alterations in the expression of tumor suppressors and oncogenes. Among them, abnormal (i.e., leaky and unorganized) tumor vasculature and the occurrence of hypoxic regions (transient and/or chronic), which are both common to all solid tumors, have been confirmed to play the most critical roles. Other hallmarks of the tumor microenvironment include the upregulation of oncogenes and DNA repair mechanisms as well as the downregulation of tumor suppressors and cell cycle regulation. Besides, the introduction of growth factor receptors and nutrient importers result in the complexation with the beta subunit of HIF to form an active transcription factor. This HIF complex binds to hypoxia-responsive elements on target genes that are always relevant to invasion, proliferation, metabolism, and drug resistance (Fig. 15.1).

Tumor-initiating cells, commonly called cancer stem cells (CSCs), represent a small proportion of cancer cells possessing the common properties of normal stem cells (SCs). CSCs can initiate new tumors after injection into animal models, which is different in other cancer cells. The small proportion of cancer cells have the function of drug resistance modulating the metastasis of cancer cells, thus resulting in the relapse of cancers by acting as an obstacle in cancer therapy. Tumor drug resistance is reported to be closely associated with CSCs because of their intrinsic or acquired properties, including the following: quiescence, specific morphology, ability to repair DNA, ability to enhance the expression of antiapoptotic proteins and drug efflux transporters, as well as ability to detoxify enzymes. Currently available radio- and chemotherapies can kill the majority of cancer cells but are usually unable to eliminate the initiating CSCs that are protected by specific resistance mechanisms. Surviving CSCs promote the growth of new tumors and metastases, resulting in cancer relapse. The recurrent tumors become even more malignant and spread more quickly. Moreover, they become resistant to previously used radiotherapy and drugs, making them more difficult to treat and leading to increased patient suffering. Different signaling pathways and genes are involved in the



- A: Hypoxic regions
- B: Leaky vasculature
- C: Discontinuous, unorganized vasculature
- D: Decreased pH and increased interstitial fluid pressure
- E: Upregulation of growth factor receptors
- F: Upregulation of ABC transporters
- G: Decreased OXPHOS, increased anaerobic metabolism
- H: Upregulation of oncogenes and anti-apoptotic factors, down-regulation of tumor suppressors and pro-apoptotic factors

Fig. 15.1 Schematic description of the selection pressures in the tumor microenvironment that leads to the development of multidrug resistance. Selection pressures such as hypoxia (A), genetic mutations in regulatory genes, and altered regulation of apoptotic factors (H) can lead to cellular adaptation and aggressive MDR characteristics. Such characteristics include increased expression of growth factor receptors (E), increased expression of drug efflux pumps (F), reversion to anaerobic metabolism (G), decreased pH (D), and increased interstitial fluid pressure (D). The abnormal vasculature in the microenvironment of tumors (B) and (C) contributes to hypoxia (selection pressure) as well as to invasion and metastasis (from [1])

maintenance of CSCs in the tumor microenvironment, which refers to a range of signaling pathways and genes. Based on the relevant phenotypes, CSCs can be characterized as a small subpopulation of cancer cells, for example, CD34⁺/CD38⁻ in leukemia cells, CD44⁺/CD24⁻ in solid tumors, and CD133⁺ in other tumors. Therefore, therapy strategies immediately applied after general cancer therapy is the most promising treatment option to achieve the goal of targeting CSCs [2].

Mechanisms of Drug Resistance in Tumors and Modulation of Drug Resistance

The characteristics of MDR include abnormal vasculature, regions of hypoxia, upregulation of ATP-binding cassette (ABC) transporters, aerobic glycolysis, and an elevated apoptotic threshold. The major course of antitumor drug resistance involves

five stages: (1) decreased drug influx, (2) increased drug efflux predominantly through ATP-driven extrusion pumps frequently of the ABC superfamily, (3) activation of DNA repair, (4) metabolic modification, and (5) detoxification and inactivation of apoptosis pathways with parallel activation of antiapoptotic cellular defense modalities. Members of the ABC superfamily, such as P-glycoprotein (Pgp/ABCB1), multidrug resistance proteins (MRPs/ABCC), and breast cancer resistance protein (BCRP/ABCG2), can act as ATP-driven drug efflux transporters by forming a unique barrier against chemotherapeutics as well as numerous endotoxins and exotoxins (Fig. 15.2).

Although these mechanisms are independent of each other and can work separately, their functions are constantly interconnected and synergistic. Cancer cells that are adapted to the reduction/loss/alteration of specific drug target, enhanced drug metabolism, and enhanced cellular repair are often resistant to a group of drugs that are similar in either structure or function. For cancer cells that are adapted to reduced drug uptake, enhanced drug efflux and drug compartmentalization alter drug accumulation within cancer cells, leading to resistance to a variety of drugs that are structurally and functionally independent [4].

Drug Delivery in Overcoming the Drug Resistance of Tumors

Various nanovehicles have been specifically designed to overcome the drug resistance of cancer cells. The drug cargo is usually released from the nanovehicle either extracellularly in the tumor or in the tumor microenvironment, i.e., the stroma and vasculature supporting the cancer cells, or intracellularly, typically through cellular uptake by receptor-mediated endocytosis [5].

Nanoparticulate systems such as liposomes, polymeric micelles, and polymer-drug conjugates have led to about two dozen clinically approved therapeutic products [6]. Other nanoparticles (NPs) that reportedly deliver therapeutic cargoes in combination include oil nanoemulsions [7], mesoporous silica NPs (MSNPs) [8], and iron oxide NPs [9, 10] (Fig. 15.3 and Table 15.1).

Using nanocarriers for the treatment of MDR is highly advantageous because they can bypass efflux by ABC transporters. Nanocarriers are internalized by non-specific endocytosis (or facilitated uptake for targeted nanocarriers), which results in higher intracellular accumulation [46]. Nanotechnology-based cancer therapy accomplishes two or more objectives in one therapeutic strategy and can dramatically improve the therapeutic index of an agent. This strategy can enable the reduction of toxicity by increasing the bioavailability, and it also converts an agent with a low therapeutic potential into a drug candidate.

Multifunctional NPs are often engineered to achieve two or more of the following objectives: drug delivery, RNAi/DNA delivery, active targeting, decreased clearance, imaging/tracking, and stimuli-responsive capabilities. To date, NPs combining a cytotoxic drug and an agent for neutralization of a well-defined mechanism of drug resistance have been tested *in vivo*, but none has reached clinical trials yet.

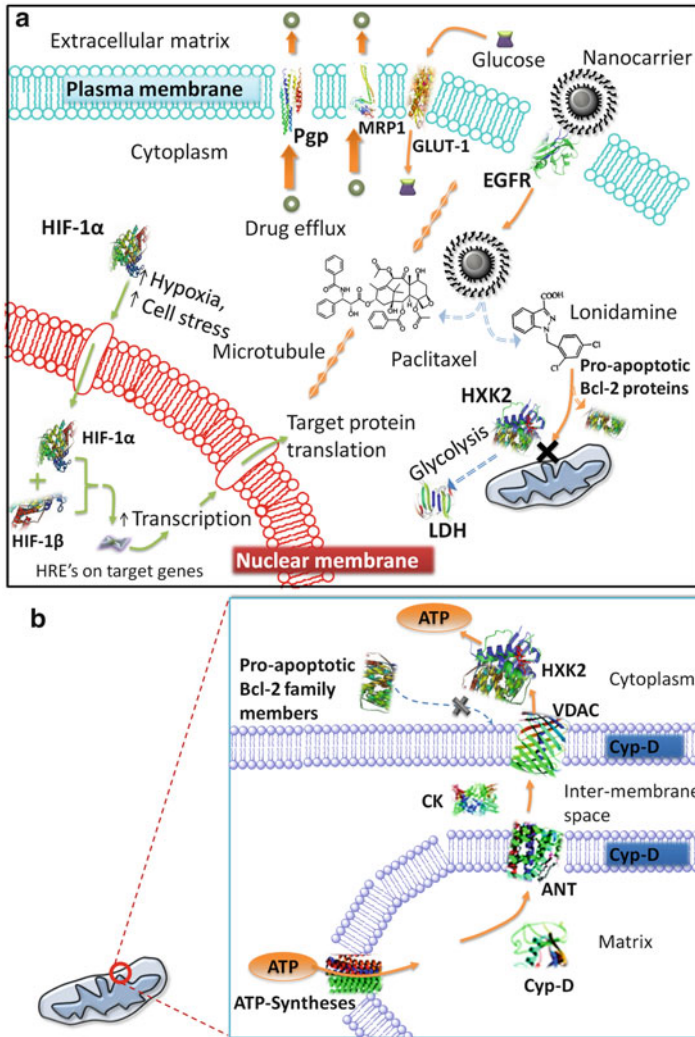


Fig. 15.2 (a) MDR characteristics and treatment strategy. HIF-1 α is located in the cytoplasm and associated with a complex of regulatory proteins under normoxic conditions. Under hypoxic conditions and cell stress, HIF-1 α translocates into the nucleus, complexes with HIF-1 β , and then binds to hypoxia-responsive elements on target genes that increase transcription and subsequent translation (e.g., EGFR). Current treatment strategies utilize a nanocarrier modified with EGFR-specific peptides to capitalize the overexpression. This receptor targeting allows facilitated uptake of the formulation, followed by the release of active agents. Receptor targeting of this nanocarrier system to the EGFR receptor should decrease residual toxicity associated with traditional chemotherapy, whereas the combination of paclitaxel with lonidamine offers a unique strategy for terminating the energy supply of MDR cancer and induce apoptosis. (b) Hexokinase 2 (HXK2) and lonidamine. This figure depicts the association of HXK2 with the components of the mitochondrial permeability transition pore complex (mtPTP) and coupling of the components to mitochondrial ATP synthase. ATP exits the mitochondria bypassing ATP synthase to the adenine nucleotide translocator (also located in the inner mitochondrial membrane), to the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane. Association of HXK2 with VDAC prevents binding of pro-apoptotic Bcl-2 family member proteins to the mtPTP (from [3])

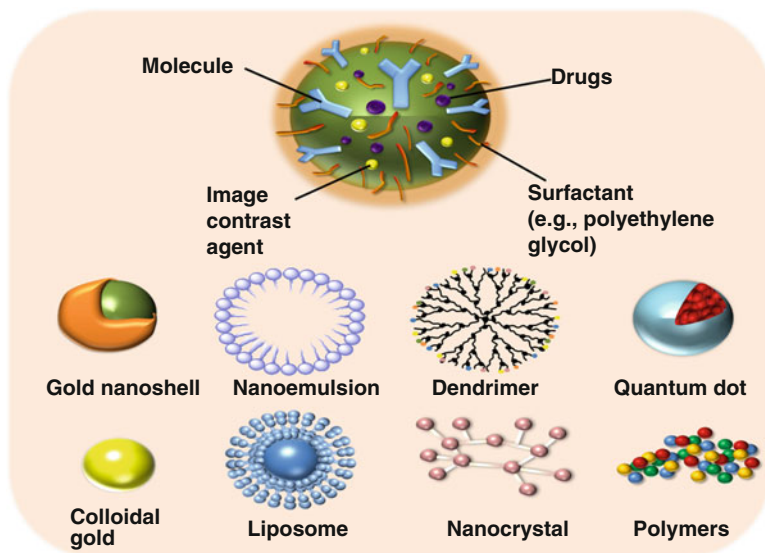


Fig. 15.3 As multicomponent multifunctional systems, nanoparticles can be functionalized with hydrophilic polymers (e.g., PEG), targeting molecules (e.g., antibodies), drugs, and imaging contrast agents. The interior core can be solid (e.g., quantum dots), liquid (e.g., liposomes), or contain an encapsulated drug (from [11])

Table 15.1 Selected examples of formulation-based therapy toward overcoming drug resistance in cancer

Nanocarrier platform	Approach to overcoming the drug resistance of tumors		
	Changing subcellular localization	Increasing intracellular availability	Targeting cancer stem cells
Nanoparticles	[12–15]	[16–19]	
Micelles		[20–24]	
Liposomes		[25, 26]	[27]
Dendrimers	[28]	[29–32]	
Polymer–drug conjugate		[33, 34]	[35]
Mesoporous silica nanoparticles	[36]	[37, 38]	
Iron oxide nanoparticles		[9, 10]	
Oil nanoemulsion		[7]	
Others	[39]	[21, 40–45]	

Nanocarriers for Tumor-Targeted Delivery

Several specific approaches are currently being explored as strategies for future cancer therapy using nanomedicines for the delivery of chemotherapeutic drugs, chemosensitizers targeting drug resistance proteins, or diagnostic aids. Accumulation

of nanocarriers at the tumor site is actually enhanced relative to the normal tissue because of the enhanced permeability and retention (EPR) effect [47]. The EPR effect results in higher accumulation of nanocarriers at the tumor site as compared with the control because of the leaky vasculature that allows passage of nanocarriers into the tumor matrix. Meanwhile, receptor targeting is also being extensively explored in experimental and clinical researches. Receptor targeting is aimed at selectively increasing the accumulation of a nanocarrier system at the tumor site by engaging a biological target that is overexpressed in cancer cells. The surface of the nanocarriers is modified with a ligand or antibody for receptor targeting, antigen targeting, or carbohydrate targeting.

The approaches to targeted nanocarrier for overcoming drug resistance are: (1) targeting the proliferating bulk of tumor cells and their intracellular compartments, (2) addressing the crosstalk between tumor cells and their microenvironment in an attempt to minimize the contribution of the stroma and vasculature to tumor cell survival and proliferation as well as to minimize drug resistance, and (3) targeting CSCs or tumor-initiating cells (TICs) [48].

Targeted delivery to the bulk of tumor cells has been extensively studied, and folate, EGFR-2 (or HER2), and transferrin are some of the most commonly used ligands. Similarly, the attachment of anti-HER2 onto NP surfaces also improves the cellular internalization of gelatin/albumin and gold NPs. Transferrin, an iron-binding glycoprotein, is a well-studied ligand for tumor targeting because of the upregulation of its receptors in numerous types of cancer. Meanwhile, various approaches aimed at targeting the microenvironment of tumor cells or the cross talk between tumor cells and their supporting stroma and/or vasculature are being developed.

Hypoxic conditions in many tumors can be potentially selected for the development of nanocarriers with redox-specific labile bonds, which can selectively target the microenvironment as well as increase drug accumulation and efficacy. Moreover, the depletion of oxygen levels in tissue (i.e., hypoxia) has long been considered as a major feature of the tumor microenvironment, which is a potential contributor to the enhanced tumorigenicity of CSCs. Targeting hypoxic factors with small interfering RNA (siRNA) or topoisomerase inhibitors are reportedly effective in overcoming drug resistance in preclinical studies. Therefore, the development of effective, systemic, and therapeutic approaches specifically focused on the tumor microenvironment is highly desirable. HIF-1 α is an attractive therapeutic target because it is a key transcription factor in tumor development and only accumulates in hypoxic tumors. Cationic mixed micellar NPs consisting of amphiphilic block copolymers poly(ϵ -caprolactone)-block-poly(2-aminoethylethylene phosphate) (PCL29-b-PPEEA21) and poly(ϵ -caprolactone)-block-poly(ethylene glycol) are suitable carriers for HIF-1 α siRNA to treat hypoxic tumors. These NPs are an excellent example of a clinical strategy of specific siRNA therapy for cancer treatment aimed at the hypoxic tumor microenvironment [49].

The concept of CSCs has been explored since the late 1930s, and these concepts have been solidified and received considerable attention in recent years. The two main aspects concerning CSCs are as follows: (1) CSCs are regular SCs that

uncontrollably grown and caused cancer and (2) CSCs arise from a subpopulation of cancer cells. In many situations, both of these concepts are rational and related to the microenvironment of a tumor. The survival and accumulation of drug-resistant CSCs following chemotherapy or radiotherapy are common explanations for the recurrence of increasingly invasive and malignant tumors. Many novel molecular targets are bound to be developed with continued in-depth research on CSCs, although it can be challenging for cancer therapy. As such, inhibiting the SC factor in MDR cells may increase the effectiveness of treatment by reducing the apoptotic threshold of these cells.

To date, the main directions in the treatment of drug-resistant cancer cells and CSC targeting are associated with four main areas. First is the design of novel gene-targeting therapies (e.g., siRNA, miRNA, and antisense oligos) against the proteins responsible for the intrinsic drug resistance and survival of CSCs, such as drug efflux transporters, antiapoptotic proteins, and members of underlying signaling pathways. Second is the development of novel and efficient small drug molecules and inhibitors, as well as polymeric drug conjugates and nanocarriers, which are able to target to the niche of CSCs. Third is the development of sensitive bio-imaging approaches, including theranostics, for the precise location of CSCs. Fourth is the potential application of physical destruction methods, such as thermoablation, photodynamic therapy (PDT), laser therapy, and surgery.

Multiple transporters have been found in CSCs, including Pgp, BCRP, and MRP. The expression of MRP1 (ABCC1) and the activity of the apoptosis inhibitor β -livin cause a high survival rate for glioblastoma CSCs after etoposide treatment. Cell surface markers expressed by CSCs/TICs are generally shared by normal somatic SCs. However, the differences between the subtle surface antigens as well as signaling pathway and metabolic alterations of CSCs/TICs and normal somatic SCs may be exploited for the selection for targeted delivery of NPs in this field. For example, the overexpression of CD44 in cancer cells is strongly linked to therapeutic drug resistance. Another marker, CD133⁺, previously found in abundance in the embryonic epithelium, is also expressed in CSCs of many cancers. Therefore, CSC targeting can be potentially applied using surface carboxylic groups. Many CSC-associated surface biomarkers, such as CD44 and CD133, can be utilized for targeting dot in anticancer therapies by vectorized nanocarriers. Recently, Wang et al. [49] designed anti-CD133 mAb-conjugated single-walled carbon nanotubes. They demonstrated that these nanotubes can selectively target CD133⁺ glioblastoma cells and assist in their photothermal destruction by a NIR laser.

The concept of “a niche” maintenance in CSCs is widely accepted by researchers because of its specific protective microenvironment as one of the intrinsic properties of CSCs. This property potentially allows them to hide in a quiescent state in tissues and avoid the damage of chemotherapy. Various physicochemical methods for the specific destruction of CSCs and the CSC-supporting environment (niche) are currently being investigated. The Notch pathway plays a critical role in the connection between angiogenesis and self-renewal of CSCs and can thus be considered as a potential therapeutic target. The niche is defined as the microenvironment where CSCs are located and where they interact with other types of cells. Evidently, the

CSC niche is a dynamic supportive system that contains a variety of cell types, cytokines, and signaling pathways. Several Notch inhibitors are being developed [50]. Mamaeva et al. [51] recently described the application of another type of nanocarriers, namely, MSNPs, for the targeted delivery of γ -secretase inhibitors of Notch signaling, which are potentially effective against CSCs.

Wnt signaling is another well-known pathway that plays a major role in embryogenesis and cancer development. Similarly, blocking the Wnt pathway in CD133⁺ colon cancer cells results in the reversal of their resistance to 5-fluorouracil [52].

Increasing Intracellular Availability and Changing Subcellular Localization

Reaching the tumor site as well as their intracellular site of action is important for therapeutics. For this phenomenon to occur, the therapeutics must escape the endosomal pathway and subsequent lysosomal degradation. Many strategies have evolved to ensure endosomal escape. A popular strategy is to modify the particles with cell-penetrating peptides that enable cell entry while evading lysosomal degradation. Another method for endosomal escape is to use pH-sensitive nanocarriers such as poly(ethylene glycol) (PEG)-modified dioleoyl phosphatidylethanolamine pH-sensitive liposomes. The pH of the intra-tumor destabilizes the liposomes, causing them to fuse with the endosomal membrane and subsequently release the cargo into the cytoplasm. Similarly, an optimized, pH-sensitive, mixed micelle system conjugated with folic acid is prepared to challenge MDR in cancers. The micelles are composed of poly(histidine-co-phenylalanine)-b-PEG and poly(L-lactic acid)-b-PEG-folate. Doxorubicin (DOX)-loaded micelles effectively kill both wild-type sensitive (A2780) and DOX-resistant ovarian MDR cancer cell lines (A2780/DOX(R)) through an instantaneous high dose of DOX in the cytosol, which results from active internalization, accelerated DOX release triggered by endosomal pH, and endosomal membrane disruption [53].

For polymer micelles, some polymers have effects on the function or expression of some efflux pump proteins. For example, Pluronic 85 (P85) can prevent the development of MDR1 phenotype in leukemia cells in vitro and in vivo as determined by Pgp expression and functional assays of the selected cells. In addition to *mdr1*, P85 alters the changes in genes implicated in apoptosis, drug metabolism, stress response, molecular transport, and tumorigenesis [54]. Meanwhile, our previous studies demonstrated that liposomes not only increase DOX levels allocated to nuclei but also extended retention in the nuclei of resistant cells [55]. Many clinical first-line anticancer drugs, such as DOX, camptothecin (CPT), and cisplatin, are DNA toxins that destroy DNA or its associated enzymes. Their cytotoxicity is maximized once they are inside the nucleus probably because of the direct damage to DNA. Thus, similar to therapeutic genes, these drugs have to localize in the nucleus to exert their pharmacological effects. For drug-resistant tumor cells, the drug can

pump out for the existence of Pgp protein. Thus, encapsulating the drug in the nanocarrier is important to overcome the function of Pgp. A polylactide-surfactant block copolymer poly(L-lactide)-vitamin E TPGS (PLA-TPGS) has been synthesized using bidentate sulfonamide zinc ethyl complex as an efficient catalyst, and its self-assembled NPs are used as carriers of DOX. The activity of Pgp in drug-resistant breast cancer MCF-7/ADR cells is found to decrease after incubation with PLA-TPGS NPs. In addition, the nuclear accumulation and cytotoxicity of DOX are significantly increased by encapsulation of the drug into the NPs [56].

Similar results are obtained using a biodegradable polymer coupled to a photosensitizer, and the resulting photosensitizer NPs are loaded with the chemotherapeutic agent DOX. The combination of photosensitizer and chemotherapeutic agent has a synergistic action on a DOX-resistant breast cancer MCF-7 cell line. This combination of photodynamic activity in a powerful nanocarrier loaded with the chemotherapeutic agent DOX can be used to deliver two types of cancer therapy simultaneously, and the addition of TPGS can further enhance the entry of DOX into the nucleus [57].

As reported, the unique and evolutionary highly conserved major vault protein (MVP) is the main component (more than 70 %) of vaults, which are ribonucleoparticles with a hollow barrel-like structure that still contains two additional proteins and vault RNAs (vRNA). Identification of MVP with human lung resistance protein, together with its upregulation in Pgp-negative chemoresistant cancer cell lines, suggests that vaults play a role in cellular detoxification processes and consequently contribute to MDR by drug sequestration or shuttling drugs from the nucleus to cytoplasmic vesicles [58]. Thus, polyamidoamine (PAMAM) dendrimers are functionalized by a polysaccharide hyaluronic acid (HA) to effectively deliver DOX as well as MVP-targeted siRNA to improve DOX chemotherapy in MCF-7/ADR cells by downregulating MVP expression. As a result, co-delivery of siRNA and DOX by PAMAM-HA exhibits satisfactory gene silencing effect as well as enhanced stability and efficient intracellular delivery of siRNA. This phenomenon allows DOX to enter into the nucleus efficiently and induce more subsequent cytotoxicity than when siRNA is absent as a result of MVP knockdown [29].

For the cell interior, the mitochondrion is the major organelle implicated in the cellular bioenergetic and biosynthetic changes accompanying cancer. These bioenergetic modifications contribute to the invasive, metastatic, and adaptive properties typical in most tumors. Moreover, mitochondrial DNA mutations are linked to the bioenergetic changes in cancer. Targeting to tumor cell metabolism or mitochondria has been proposed as a novel strategy for the treatment of tumor. The most important aspect in the physiology of cancer is the role of mitochondria in energy metabolism and cell cycle regulation. Strong evidence supports the rationale for the development of anticancer strategies based on mitochondrial targets. Mitochondria play a key role in the complex apoptotic mechanism and trigger cell death through several mechanisms, such as disrupting electron transport and energy metabolism, releasing or activating proteins that mediate apoptosis, and altering the cellular redox potential.

Nanotechnology, which encompasses materials and methods at the nanoscale, is an attractive approach to designing mitochondrial therapeutics that either target or avoid mitochondria. Nanosystems that target mitochondria can enhance efficacy in treating mitochondrial diseases, whereas those that avoid mitochondria may help reduce mitochondrial toxicity. The surface modification of nanocarriers can also be tailored to achieve subcellular localization such as mitochondrial targeting, which is often achieved using mitochondrial leader sequences or the negative membrane potential of mitochondria through the use of “mitochondriotropics.” Mitochondriotropics are molecules that have delocalized positive charges such as triphenyl phosphonium [59].

For mitochondrial targeting, the selective accumulation of Au NPs in the mitochondria of cancer cells has been reported [60]. Their long-term retention decreases the mitochondrial membrane potential and increases the reactive oxygen species level that enhances the likelihood of cell death. Taking advantage of the development of SV30, a new analog of the pro-apoptotic molecule HA14-1, 57 nm organic solvent-free lipid nanocapsules loaded with SV30 (SV30-LNCs) are formulated using an inversion phase process. Encapsulated SV30 is found to improve mitochondrial targeting, which may elicit considerable interest toward the development of mitochondrion-targeted nanomedicines [61]. In addition, the known mitochondriotropic ligand triphenyl phosphonium (TPP) has been conjugated on the surface of a dendrimer. A fraction of the cationic surface charge of G(5)-D is neutralized by partial acetylation of the primary amine groups. The newly developed TPP-anchored dendrimer (G(5)-D-Ac-TPP) is efficiently consumed by the cells and demonstrates good mitochondrial targeting [62].

Combination Therapy Toward Overcoming Drug Resistance

Many combinatorial NP formulations have been successful in reversing MDR *in vitro* and *in vivo* of cancer models by co-delivering chemosensitizing agents and chemotherapy agents. Among many cellular mutations that diminish the effectiveness of anticancer drugs, the overexpression of multidrug transporters and altered apoptosis are the two underlying mechanisms by which cancer cells acquire resistance to multiple structurally and mechanistically unrelated drugs. NPs of 10–200 nm in diameter have shown more favorable antitumor pharmacokinetic profiles than small-molecule drugs. These drug-loaded NPs exhibit prolonged systemic circulation lifetime, sustained drug release kinetics, and advanced tumor accumulation [6]. Various NP platforms such as liposomes, polymeric micelles, dendrimers, nanoemulsion, and mesoporous silica particles have been used to carry broad classes of therapeutics, including cytotoxic agents, chemosensitizers, siRNA, and antiangiogenic agents (Fig. 15.4 and Table 15.2).

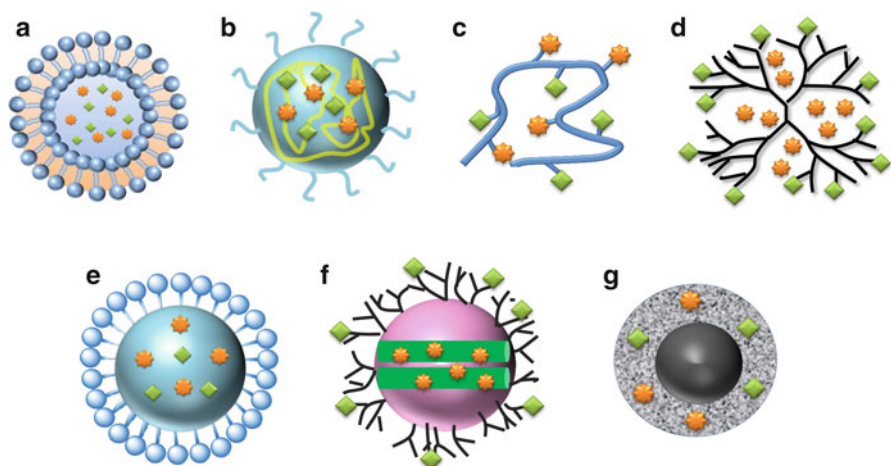


Fig. 15.4 Schematic of nanoscale drug carriers used for combinatorial drug delivery: (a) liposome, (b) polymeric micelle, (c) polymer–drug conjugate, (d) dendrimer, (e) oil nanoemulsion, (f) mesoporous silica nanoparticle, and (g) iron oxide nanoparticle (from [63])

Combination of Drug Delivery and Drug Efflux Modulation

Drug resistance is considered to be the main reason for therapeutic failure in advanced cancer treatment. In many cases, drug transporter proteins (e.g., Pgp and MRP) that can pump out the intracellular drug are always overexpressed in most drug-resistant cancer cell lines. These drug transporter proteins are some of the most extensively characterized barriers to chemotherapy. Accordingly, a number of nanocarriers have been designed to sensitize drug-resistance tumor cells because they can aid in drug escape from the transporters, inhibit ATPase activity, or indirectly deplete cellular ATP, thereby leading to enhanced intracellular accumulation of therapeutic agents [68].

During chemotherapy, one of several ABC drug transporters, such as Pgp, MRP1, or ABCG2, becomes upregulated in some cancer cells. This phenomenon causes insensitivity to drugs and, subsequently, drug resistance. To date, the genes for 48 ABC proteins have been identified in the human genome and subdivided into seven families (ABC A–G) based on structural and sequential similarities. The decrease in intracellular drug accumulation is always caused by an undetermined energy-dependent, carrier-mediated mechanism. Not until 1976 was a 170 kDa cell membrane glycoprotein named Pgp discovered, and its link to the MDR phenotype was confirmed by Juliano and Ling [88]. In addition to Pgp (ABCB1), MRP1 (ABCC1), and ABCG2, at least 12 other ABC transporters are currently linked to MDR or can cause reduced intracellular drug accumulation.

Attempts on inhibitor-based chemosensitization and on the identification of new inhibitors of ABC transporters are currently ongoing. A large number of cancer-treating drugs have been identified as substrates of Pgp, including Vinca alkaloids,

Table 15.2 Selected examples of formulation-based combination therapy toward overcoming drug resistance in cancer

Nanocarrier platform	Combined or sequential treatment	Main bioactive or cytotoxic cargo	Tumor type	Approach to overcoming drug resistance of tumor	Ref.
Nanoparticles	Curcumin	Doxorubicin	K562/ADR cells	Modulation of drug efflux and apoptotic threshold	[64]
	Londamine	Paclitaxel	SKOV3-TR cells and the MDA-MB-435 cells	Modulation of apoptotic threshold	[3]
	Bcl-2 siRNA	Cisplatin	Hep-2 cells and HeLa cells	RNA interference therapy	[65]
	Photodynamic therapy	Doxorubicin	NCI-ADR-RES cells	Combination drug and energy	[66]
	Tariquidar	Paclitaxel	JC and NCI-ADR-RES cells	Modulation of drug efflux	[67]
	Pgp siRNA	Paclitaxel	JC cells	RNA interference therapy	[68]
	Survivin shRNA	Paclitaxel	A549/ADR cells	RNA interference therapy	[69]
	Curcumin	Nutlin-3a	Y79 cells	Modulation of apoptotic threshold	[70]
	Idarubicin	Doxorubicin	P388/ADR cell lines	Modulation of drug efflux	[71]
	MDR-1 siRNA	Paclitaxel	SKOV3 _{TR} cells	Modulation of drug efflux	[72]
	Mitomycin C	Doxorubicin	MDA-MB-435/LCC6/MDR1 cell line	Modulation of drug efflux	[73]
	Fluorodeoxyuridine	Cytarabine	HepG2 cells	Modulation of drug efflux	[74]
	Photosensitizer	Doxorubicin	MCF-7/ADR cell line	Combination drug and energy	[57]
	C6-ceramide	Paclitaxel	SKOV-3 xenografts	Modulation of apoptotic threshold	[75]
	Tamoxifen	Paclitaxel	SKOV3 _{TR} cells	Modulation of apoptotic threshold	[76]
	Verapamil	Vincristine	MCF-7/ADR cell line	Modulation of drug efflux	[77]

(continued)

Table 15.2 (continued)

Nanocarrier platform	Combined or sequential treatment	Main bioactive or cytotoxic cargo	Tumor type	Approach to overcoming drug resistance of tumor	Ref.
Liposomes	MRPI siRNA and BCL-2 mRNA	doxorubicin	H69AR/MDR, MCF-7/ADR and HCT15 cell	RNA interference therapy	[78]
	Amlodipine Quinacrine	Topotecan Vincristine	HL-60/MDR cell line Drug resistant human leukemia cells	Modulation of drug efflux Modulation of drug efflux	[79] [80]
Micelles	Tariquidar	Paclitaxel	SKOV-3 cell line	Modulation of drug efflux	[81]
	Verapamil	Doxorubicin	K562 leukemia cells	Modulation of drug efflux	[82]
	Photosensitizer	Doxorubicin	MCF-7/ADR cell line	Combination drug and energy	[83]
Mesoporous silica nanoparticles	MDR-1 siRNA	Doxorubicin	MCF-7/ADR cell line	Modulation of drug efflux	[84]
	P-Glycoprotein siRNA	Doxorubicin	KB-V1/ADR cells	RNA interference therapy	[85]
	MRPI and BCL2 mRNA	Doxorubicin and cisplatin	A549 cell line	RNA interference therapy	[86]
	Bcl-2 siRNA	Doxorubicin	A2780/AD cells	Modulation of apoptotic threshold	[8]
Iron oxide nanoparticles	Rapamycin	Paclitaxel	MCF-7/ADR cell line	Modulation of drug efflux	[9]
Dendrimers	MVP siRNA	Doxorubicin	MCF-7/ADR cells	RNA interference therapy	[29]
	Curcumin	Paclitaxel	SKOV _{3-TR} cells	Modulation of apoptotic threshold	[7]
Gold nanorods	ASCL1 siRNA	Doxorubicin	NE cancer cells	RNA interference therapy	[87]

taxanes, etoposide, teniposide, colchicines, actinomycin D, CPTs, imatinib mesylate, saquinavir, methotrexate, and mitoxantrone. An appealing approach to overcoming MDR is the co-administration of a chemotherapeutic agent and a Pgp inhibitor. Nanocarrier systems containing a combination of cytotoxic drugs and efflux pump inhibitors, such as cyclosporine, verapamil, and tariquidar, have been used to suppress the MDR effect. The first attempt to co-deliver a chemosensitizer with chemotherapeutics in a single nanocarrier was a polyalkylcyanoacrylate NP system loaded with Pgp inhibitor cyclosporin A (CyA) and DOX [89]. Against a DOX-resistant leukemia cell line (P388), the co-encapsulation of CyA and DOX induces nearly a twofold increase in toxicity compared with DOX-only NPs. The enhanced efficacy is not observed when free CyA is applied with the DOX-only NPs. This finding suggests that the NP-coordinated delivery of two bioactive agents is essential for their cooperative activity. Various drug delivery nanovehicles are engineered to evade or overcome drug extrusion by drug efflux transporters, thereby resulting in enhanced chemotherapeutic drug accumulation in the cytosol and/or the nucleus of cancer cells and consequent elimination of tumor cells. These nanovehicles include oil nanoemulsions, polymeric micelles, liposomes, copolymeric NPs conjugated to quantum dots, and metallic NPs.

Permanent elimination or deactivation of any ABC transporter is unrealistic and unreasonable because of their important physiological and pharmacological roles in the human body. However, MDR in cancer caused by the overexpression of ABC drug transporters can be transiently modulated by various means, including direct inhibition, gene silencing, transcriptional regulation, and drug encapsulation. However, no clinically applicable inhibitor of ABC transporters exists to date. The reason for the unsuccessful clinical trials is complex but may be predominantly due to the unfavorable toxicity of inhibitors. MDR in cancer is apparently caused by multiple mechanisms that operate either independently or in unison. Overexpression of drug transporters is just one of the many reasons that cancer cells have adapted to survive the diversity of agents used in cancer chemotherapy. Over 30 years has passed since the discovery of Pgp in 1976, yet no simple and feasible solution to overcoming MDR in cancer has been discovered. The complexity and identification of new MDR-linked ABC transporters produce more challenges. Nevertheless, based on the new discoveries and advancements made on the identification, biological characterization, and structural analysis of MDR-linked ABC transporters over the years, we are one step closer to understanding clinical MDR in cancer.

Combination Drug Delivery and Modulation of Apoptotic Threshold

Cell apoptosis requires a minimum cellular threshold to be overcome. In cancer cells, this threshold is elevated to the extent that extracellular and intracellular insults sufficient in inducing apoptosis in normal cells have no effect. MDR cells have developed various mechanisms for increasing their apoptotic threshold. Decreased ceramide levels and the Warburg effect are the two major mechanisms that MDR cells utilize to increase their apoptotic threshold. The response to MDR

is associated with alterations in the apoptosis pathways. Therapeutic NPs have been developed to co-encapsulate compounds that repair the dysfunctional apoptotic signaling. One example of such pro-apoptotic compound is ceramide, which is produced by cells under environmental stress and serves as a key messenger in programmed cell death.

An increasing number of studies have implicated ceramide, sphingosine-1-phosphate, as well as the genes involved in their biosynthesis, catabolism, and signaling, in various aspects of oncogenesis, cancer progression, as well as anticancer drug resistance and radiation resistance. Based on these findings, several research groups have used the strategy of inducing elevated levels of ceramide to decrease the threshold of apoptotic signaling in MDR cells while simultaneously delivering a cytotoxic drug (e.g., paclitaxel) using polymeric NPs.

A polymeric micelle formulation based on poly(ethylene oxide)-poly(epsilon-caprolactone) (PEO-PCL) for co-delivering exogenous ceramide and paclitaxel to address ceramide metabolism has been developed [90]. Against a paclitaxel-resistant ovarian cancer cell line (SKOV-3TR), the combinatorial formulation is found to increase the paclitaxel sensitivity of MDR cells to the same level as non-MDR cells. Combination with ceramide shows a 100-fold increase in efficacy compared with paclitaxel-only NPs. In another study, polymeric blend NPs have been prepared for the co-encapsulation of paclitaxel and C6-ceramide (CER), a synthetic analog of ceramide [75]. In vivo studies indicate that combination therapy with NPs harboring both paclitaxel and CER can enhance apoptotic signaling and reduce the tumor volume at least twofold compared with traditional standard paclitaxel monotherapy [75]. Yet another approach to increasing intracellular ceramide is the use of siRNA to silence glucosylceramide synthase. This strategy decreases the expression of Pgp in MDR cells, verifying the significance of ceramide in apoptotic modulation [91].

Combination Drug Delivery and Intracellular pH Modulation

The decreased pH associated with MDR cells has been utilized in many strategies for overcoming MDR. Some strategies are aimed at altering intracellular pH; others make use of pH-sensitive constituents to control the release of drugs. Novel pH-responsive polymers such as poly(β -amino ester), soluble below pH 6.5, are incorporated into NP formulations to localize the release of therapeutic agents in the acidic cellular environment of tumors and subcellular endosomal/lysosomal compartments.

Drugs encapsulated in pH-sensitive polymeric micelles have also been developed to target MDR cancer. Zwitterionic oligopeptide liposomes (HHG2C(18)-L) containing a smart lipid (1,5-dioctadecyl-L-glutamyl 2-histidyl-hexahydrobenzoic acid, HHG2C(18)) have been developed to overcome the barriers faced by anticancer drugs on the route from the site of injection into the body to the final antitumor target within transport steps with multiple physiological and biological barriers. HHG2C(18)-L shows a multistage pH response to the tumor cell (the mitochondria

in this case). Their multistage pH response leads to more effective entry of anticancer agents into the tumor cell, improved escape from the endolysosomes, and accumulation in the mitochondria [92].

Nanocarriers for Combination Drug and Energy

Any organ heated to temperatures between 41 and 46 °C is defined as hyperthermia. Hyperthermia leads to reversible cell damage; however, when used as an adjunct treatment, it can help increase the efficacy of chemotherapy and enhance radiation-induced tumor damage. Hyperthermia has been utilized to change the morphology of a tumor to enhance the delivery of polymeric and liposomal NPs by increasing the blood flow to the tumor. It has also been successfully combined with DOX-loaded liposomes that target the folate receptor of tumor cells [93]. These temperature-sensitive systems can be designed to release drug payloads in the presence of specific temperature triggers.

In addition, clinical improvements to ultrasound focusing are being developed to improve the control and precise targeting of ultrasonic waves [94]. Combining localized ultrasound with nanocarrier therapies can exert a dramatic effect on the reduction of the residual toxicity associated with chemotherapy. Meanwhile, PDT is a form of cancer treatment that involves the use of photosensitizers as therapeutic agents. Under light irradiation, photosensitizers enter a triplet state of excitation. This triplet state of energy is easily transferred to oxygen molecules, which are subsequently converted into reactive oxygen species that are capable of damaging cells [95]. This method of treatment has high selectivity because only the cells exposed to both light and photosensitizer are affected.

RNA Interference to Overcome MDR

The clinical applications of small-molecule drugs that inhibit Pgp are not all successful. Hence, therapeutic strategies using RNA interference technology to overcome MDR are actively being explored. siRNA is a short double-stranded RNA that shows specific and effective gene silencing activity by the sequence-specific downregulation of a complementary messenger RNA. Therapeutic applications of siRNA have been limited because of their rapid enzymatic degradation by ribonuclease activity in serum and poor cellular uptake by passive diffusion [96]. The reversibility of the MDR phenotype of human cancer cells through the activation of the RNAi pathway by knocking down the MDR1/Pgp encoding mRNA was first reported in 2003 [97].

Drug efflux transporter genes that are being targeted include ABCB1 (MDR1/Pgp) and ABCC1 (MRP1), and these genes have been studied for decades. Gene silencing may be achieved at the mRNA level using siRNA constructs or antisense oligodeoxynucleotides (asODNs), which results in decreased MDR1 expression. Various drug delivery carriers for the targeted silencing of drug resistance genes

have been described, including liposomes and different polymers, typically of cationic nature such as chitosan and its derivatives. A micellar system consisting of degradable poly(ethylene oxide)-block-poly(ϵ -caprolactone) (PEO-b-PCL) block copolymers with functional groups on both blocks has been prepared. The functional group on the PCL block is used to incorporate short polyamines for complexation with siRNA or to chemically conjugate DOX using a pH-sensitive hydrazone linkage. This system is used to improve the efficacy of DOX in multidrug-resistant MDA-MB-435 human tumor models that overexpress Pgp. The improvement is carried out by the simultaneous intracellular accumulation of DOX and siRNA against Pgp expression [98].

Targeting MDR1 gene transcripts has also been developed by harnessing bacterium-derived minicells encapsulating specific siRNA duplexes and chemotherapeutics [99]. Minicells targeted by specific antibodies to surface receptors of tumor cells are then used to deliver synergistic cargoes to tumor xenografts with high specificity.

Among the mechanisms of drug resistance independent of drug efflux pumps that have been targeted with an NP approach, some modalities are related to the Bcl2 and HIF1 α genes. Bcl2 family proteins are regulators of programmed cell death (particularly apoptosis), and the HIF1 α gene encodes for a transcription factor that plays a key role in the cellular response to hypoxia. Gene silencing is performed using siRNA or as ODNs. MSNs are utilized for the simultaneous delivery of Dox and Bcl2 siRNA [8]. Dox-loaded MSNs modified with amine-terminated PAMAM dendrimers facilitate conjugation with Bcl2 siRNA. Moreover, the simultaneous delivery of Bcl2 siRNA significantly suppresses Bcl2 mRNA and efficiently overcomes the MDR phenotype presumably using an inhibitory activity that these PAMAM dendrimer-based NPs exert on Pgp-mediated drug efflux [8].

A chemotherapeutic agent (DOX) and Pgp siRNA can be co-encapsulated by MSNPs and transported to a drug-resistant cancer cell line (KB-V1 cells), subsequently accomplishing cell killing in an additive or synergistic fashion [85]. Although a number of research have reported the RNAi modulation of cancer MDR in vivo, the lack of an efficient delivery strategy for administering shRNA to cancer patients is the major drawback. Various strategies have been explored but with no successful results. These studies demonstrate that a more efficient mode of delivery and nanocarriers, which are a promising platform for the efficient delivery of RNAi, is important in the clinical application of RNAi.

Conclusion

The unsatisfactory therapeutic effect of chemotherapy in treating solid tumors is multifactorial, and the occurrence of clinical tumor drug resistance is usually caused by a complex and unknown mechanism. Moreover, solid tumors are heterogeneous, structurally complex, and contain different kinds of cell. To the best of our knowledge,

although various nanocarrier platforms for targeted delivery of anticancer drugs have already undergone in vivo testing in animal models and clinical evaluation in humans, no reports exist on NPs for the delivery of drug combinations aimed at overcoming drug resistance. The development of appropriate combinations of chemotherapies and nanotherapies, including novel gene-silencing, drug efflux-inhibiting, and CSC-targeting strategies, are the most effective methods of treating drug-resistant and aggressive tumors.

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