Therapeutic Nanoparticles to Combat Cancer Drug Resistance

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Abstract: This review focuses on the application of drug-loaded nanoparticles (NPs), also called therapeutic NPs, to combat cancer chemoresistance. Many cancer patients have encouraging response to first line chemotherapies but end up with cancer progression or cancer recurrence that requires further treatment. Response to subsequent chemotherapies with various agents usually drops significantly due to formidable cancer chemoresistance. A number of mechanisms have been postulated to account for cancer chemoresistance or poor response to chemotherapy. The best studied mechanism of resistance is mediated through the alteration in the drug efflux proteins responsible for the removal of many commonly used anticancer drugs. Therapeutic NPs have emerged as an innovative and promising alternative of the conventional small molecule chemotherapies to combat cancer drug resistance and have shown enhanced therapeutic efficacy and reduced adverse side effects as compared to their small molecule counterparts. Here the possible mechanisms of therapeutic NPs to combat cancer chemoresistance are reviewed, including prolonging drug systemic circulation lifetime, targeted drug delivery, stimuli-responsive drug release, endocytic uptake of drugs and co-delivering chemo-sensitizing agents. We also call attention to the current challenges and needs of developing therapeutic NPs to combat cancer drug resistance.

Keywords: Cancer chemoresistance, nanoparticle drug delivery, long circulation, targeted delivery, stimuli-responsive drug release, endocytic uptake, combination therapy.

INTRODUCTION

Cancer drug resistance is one of the biggest challenges in clinical cancer treatment with anticancer drugs [1]. Over the course of chemotherapy, cancer cells commonly develop defense mechanisms against the treatment, leading to therapy failure and tumor relapses. The acquisition of cancer drug resistance can be attributed to inefficient drug delivery to tumor tissues and tumor cells, which results in low drug concentrations at the tumor sites and incomplete treatment. Since anticancer drugs are typically toxic toward healthy proliferating cells as well, drug dosage must be restricted to avoid potentially lethal side effects. Therapeutic efficacy of such restricted drug dosage is further diminished by factors such as poor pharmacokinetics of the drugs, including limited systemic circulation lifetime, undesirable biodistribution and non-specific cellular uptake, and poor tumor vascularity that limits drug accessing to tumor tissues [2]. As a result each dose of chemotherapy treats the tumors partially and additional courses are required to eliminate the remaining tumors. Surviving cancer cells are therefore subject to a selective pressure that favors genetic mutations toward drug resistance. After repeated treatments, cancer cells that are initially vulnerable to certain drugs might stop responding to them. In such cases the chemotherapy is rendered ineffective and either higher drug dosages or new therapeutic agents are necessary to continue the treatment.

In developing drug resistance, cancer cells undergo genetic mutations and changes in signaling pathways that interfere with the action mechanisms of a drug. Once cancer cells acquire resistance against certain drugs, they are desensitized to structurally and functionally similar drugs as well. One strategy to overcome cancer drug resistance is concurrently using multiple therapeutic drugs that are functionally and mechanistically distinct to treat the same tumors. A combination of drugs with different cellular targets may create a higher revolutionary hurdle for cancer cells to develop a defense mechanism. Some clinical data has demonstrated that combination chemotherapy has better efficacy compared to single-drug alternatives [3, 4]. However, current combination chemotherapy still fails in many cases because cancer cells can exhibit simultaneous resistance toward multiple functionally unrelated drugs. Such phenomenon, known as multi-drug resistance (MDR), is associated with alterations in apoptotic signaling, enhanced damage repair mechanisms, and an overexpression of drug-efflux pumps [1]. Of these, overexpression of drug-efflux pumps is the most common MDR mechanism. These efflux pumps are transmembrane transporters that bind to different types of drug molecules as they enter the plasma membranes, followed by an ATP-mediated protein shape change to release the drugs into the extracellular space. Therefore, overexpression of multidrug transporters enables a rapid efflux of anticancer drugs out of the tumor cells. P-glycoprotein (P-gp) is one of the most frequently expressed multidrug transporters. It is present in many types of cancers such as gastrointestinal, liver, pancreatic, and ovarian cancers [5-8]. In clinical cancer treatment, P-gp severely limits the therapeutic options as it targets many widely used anti-cancer drugs including doxorubicin, vinblastine and taxol.

To combat cancer drug resistance and effectively treat cancers, strategies on both developing novel pharmaceuticals and delivering existing drugs to tumor sites in more effective manners have been extensively studied. From the drug delivery perspective, cancer drug resistance can be suppressed by improving drug delivery to the tumor sites and reducing MDR-based drug efflux. A plausible way for efficient cancer drug delivery is to associate anticancer drugs with NPs. In the last a few decades, the advancement of nanotechnology has made possible the synthesis of nanoscale, biocompatible and biodegradable drug delivery vehicles. Many types of nanocarriers including liposomes, solid lipid NPs and polymeric NPs have been developed to deliver a variety of drugs [9-11]. These nanocarriers have demonstrated desirable drug delivery characteristics such as prolonged systemic circulation lifetime, reduced non-specific cellular uptake, targeting abilities, controllable drug release, and multidrug encapsulation for combinatorial treatment. Recently, NPs with a size range of 50~150 nm are emerging as a promising drug delivery platform for cancer treatment. Numerous chemotherapeutic drugs, including many that are otherwise insoluble in the blood, have been successfully encapsulated in NPs. A number of NP-based cancer drugs that are currently on the market or in clinical trials have been reviewed by Peer et al [12]. This paper will provide a perspective on the strengths of NPs in tackling cancer drug resistance. More specifically, as illustrated in Fig. (**1**), this review will first describe the advantages of NPs in facilitating drug delivery to tumor sites and then discuss how NPs can combat cancer drug resistance mechanisms on the cellular level.

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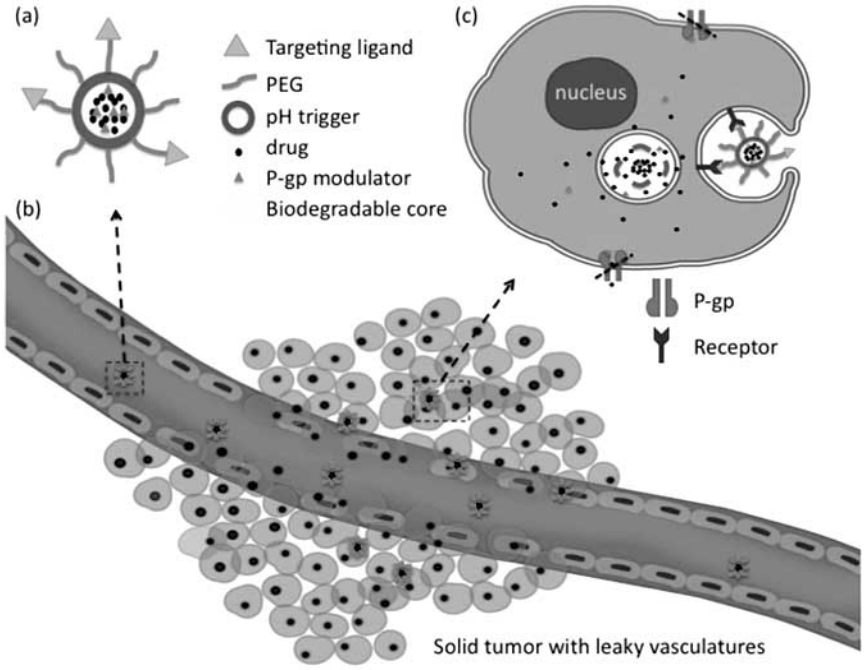

Fig. (1). Schematic illustrations of NP drug delivery to cancer. **(a)** Key features of drug delivery NPs. **(b)** Long circulating NPs accumulate at tumor sites through the enhanced permeation and retention (EPR) effect. **(c)** Receptor-mediated cellular uptake of therapeutic NPs and drug release inside the cells.

PROLONGED DRUG SYSTEMIC CIRCULATION LIFE-TIME

In vitro studies have shown that highly concentrated anticancer drugs can kill cancer cells that exhibit MDR phenotype [13-16]. It has been hypothesized that any MDR mechanisms can be overwhelmed by high doses of drugs [16]. Since anticancer drugs are toxic to healthy tissues, which restricts the allowable drug dosage, preferential drug accumulation at tumor sites is essential in overcoming MDR. Tumor vessels are highly abnormal because they lack adequate pericyte coverage, branch and anastomose abnormally, lack functional receptors for angiotensin II and contain large fenestrations that render them highly porous. This abnormal porosity is the basis for the "enhanced permeability and retention" (EPR) [17-19] effect by which small molecule anticancer drugs and high molecular weight substances such as therapeutic NPs can escape from tumor capillaries into the extracellular tumor matrix where they are selectively retained in part due to the lack of well developed lymphatics in tumors. However, the EPR effect has negligible benefit for systemically administered free anticancer drugs because of their short circulation lifetime. These small molecule drugs are rapidly removed from the blood by non-specific cellular uptake, immune opsonization, plasma degradation, glomerular filtration and hepatic clearance before they reach the tissues and cells of interest. In general, small molecule anticancer drugs are cleared from the blood within hours or shorter after administration. To extend drugs' systemic circulation lifetime, NPs have been proposed to carry and deliver drugs.

Early attempts to prolong drug systemic circulation using liposomes, spherical lipid vesicles consisting of a bilayered lipid membrane [20], were met with marginal success. By loading drugs to the hydrophobic lipid membranes or the aqueous interior of liposomes, the drug payloads were shielded from renal filtration and plasma degradation but the liposomes failed to escape from the uptake by the reticuloendothelial system (RES) such as monocytes and macrophages. Such RES uptake led to rapid removal of the liposomal drugs from the blood. The development of stealth NPs was a major breakthrough in prolonging circulation time while minimizing non-specific cellular uptake. These long-circulating NPs were typically coated with a layer of polyethylene glycol (PEG), which is a synthetic hydrophilic polymer. The PEG coating forms a hydration layer that retards RES recognitions by sterically inhibiting hydrophobic and electrostatic interactions with plasma proteins. Many studies characterized the effects of PEG length and density on NP systemic circulation time and biodistributions [21-23]. It has been reported that NPs coated by PEG with a molecular weight of 2~5 kDa give desirable circulation profile for medical applications. It has also been reported that NP size significantly affects their *in vivo* delivery performance. A particle diameter between 50 to 150nm is optimal for drug retention and tumor extravasation [24, 25].

Clinical studies have revealed a striking circulation lifetime difference between free drugs and their NP-encapsulated counterparts. In a detailed review, Gabizon *et al.* have compared the pharmacokinetics between free doxorubicin (Dox) and pegylated liposomal Dox (Doxil) [26]. Doxil showed improved pharmacokinetic profiles in both human and animal studies. For a 50 mg/m^2 dose injection in human, drug availability of Doxil in blood represented by the area under the concentration (AUC)-time curve was about 300-fold as high as free Dox. More importantly, the enhanced drug retention in the blood was translated to higher and more preferential tumor uptake. For example, in a study in AIDS-related Kaposi's sarcoma, patients treated with Doxil showed 5- to 11-fold higher drug concentration in skin tumor lesions compared to those treated with free Dox. The lesions also contained 10- to 15-fold higher drug concentration than that in adjacent normal skin, suggesting passive targeting due toEPR effect [27, 28]. Visualization of radio-labeled pegylated liposomes further confirmed their ability to localize at tumor sites. Other types of pegylated NP systems have shown similar advantages in preclinical studies. By prolonging the circulation time, NPs can more effectively extravagate out of the tumor vasculatures and facilitate preferential drug delivery.

The enhanced tumor accumulation of therapeutic NPs has great implications in addressing cancer drug resistance. For instance, high extracellular drug concentration enhances drug diffusion through cellular membrane and could saturate P-gp based drug efflux. Endocytosis of therapeutic NPs can potentially suppress the P-gp mechanism as well which will be described with more details in the following section. Resistance due to mutated and less effi-

cient drug targets could also be overcome by the higher number of drug molecules. Furthermore, excess chemotherapeutic agents could inflict significant cell injuries and induce cell death even if the cell has dysfunctional apoptotic processes. In fact, overwhelming MDR with high drug dosage is the underlying theory behind high-dose chemotherapy followed by bone marrow rescue [16]. Because of its selective tumor uptake, NP drug delivery may offer the potency of high-dose chemotherapy without the associated risks and side effects.

TARGETED DRUG DELIVERY

Passive targeting through EPR effect has been best observed in small and well vascularized tumors. In poorly vascularized tumors such as that of colon and pancreas, passive tumor accumulation is often inadequate. Also, vessel permeability might differ within a single solid tumor, resulting in non-uniform drug profusion and incomplete cancer treatment. This limitation has motivated enormous efforts in achieving active targeting through ligand-receptor interactions. Targeting ligands such as antibodies, aptamers, peptides and carbohydrates can be covalently conjugated to NP surfaces. Depending on the size of the NPs and the ligands, tens to hundreds of targeting ligands can be incorporated to each NP to enable multivalent targeting ability that enhances the overall strength of NP binding to the target tumor cells.

One common approach in preparing targeted NPs takes advantage of the well-known molecular recognitions in antibody-antigen binding. Certain antigens are overexpressed in specific cancer types and antibody-modified NPs have shown improved accumulation at those tumor sites. For instance, anti-HER2 immunoliposomes yielded 700-fold higher drug uptake compared to non-targeted liposomes in HER2-overexpressing breast tumors [29]. Not only did targeted liposomes deliver the drugs to the tumor site, they also facilitated intracellular drug delivery through receptor-mediated endocytosis. After intravenous injection, the anti-HER2 liposomes were found mostly in the cytoplasm of cancer cells whereas the bare liposomes accumulated extracellularly or in macrophages. The study demonstrated superior therapeutic efficacy for targeted NPs. Other antibodies that have been examined for NP cancer targeting include CC52 antibody-modified liposomes against colon adenocarcinoma [30], anti-CD19 for B cell lymphoma [31], and 34A antibody for metastatic lung cancer [32].

Because antibodies are relatively large (~150KDa in molecular weight), their conjugation often results in poor size control and reduced stealth capability. These shortcomings led to the emergence of alternative targeting ligands. Such ligands include variations of whole antibodies such as Fab fragments [32] and single chain variable fragments [33], growth factors and nutrients whose receptors are overexpressed in cancer cells [34, 35], RNA-based aptamers [36], and peptides such as RGD and LyP-1 that target tumor vasculatures [37-39]. The small physical dimension of these alternatives enables high ligand density and more effective multivalent targeting without compromising the particle's circulation time. For example, in an *in vivo* study that compared the performance of antibody-liposomes to their Fab counterparts, the Fab-liposomes showed a 6-fold increase in circulation half-life and a 2-fold increase in tumor retention [32].

STIMULI-RESPONSIVE DRUG RELEASE

In addition to long circulation half-life and active targeting, stimuli-responsive drug release is another unique feature of some therapeutic NPs that can help suppress MDR of cancer cells. The drug release rate of therapeutic NPs determines their therapeutic efficacy. Fast release kinetics result in premature drug loss while the NPs are circulating in the blood stream. Slow release kinetics, on the other hand, may fail to outcompete the drug efflux mediated by P-gp and lead to tolerance by the resistant cancer cells. For an ideal therapeutic NP, negligible amount of drugs will be lost during the circulation period while rapid intracellular drug release occurs after the NPs are internalized by the target cancer cells. To achieve the optimal differential release kinetics, environmentally responsive triggers have been investigated to cause sudden particle destabilization under certain stimuli. In cancer drug delivery, pH-sensitive triggers are the most widely studied because tumor sites are characterized by acidic microenviornments (pH =6~6.8) [40]. In addition, endosomes and lysosomes have even higher acidity (pH=5~6) [41] that can facilitate selective drug release once the NPs are endocytosed.

Several schemes of pH-triggered drug release have been implemented on NP drug delivery systems. One example is acid-responsive liposomes whose membrane structure becomes leaky in acidic environments [42]. These liposomes usually contain phosphatidylethanolamine (PE) and other stabilizing amphiphiles. At physiological pH (pH=7.4), the deprotonated and negatively charged amphiphiles are intercalated among PE molecules and stabilize the bilayer structure. As pH drops, amphiphilic molecules are protonated and lose their stabilizing effect, leading to liposome destabilization. Some of the mildly acidic amphiphiles that give rise to the pH-sensitive trigger include cholesteryl hemisuccinate, oleic acid, and hydrophobized alkylated N-isopropylacrylamide copolymers [42-44].

More recently, pH-sensitive micelles (PHSM) have been designed. These polymeric micelles contain poly(L-histidine)–poly(ethylene glycol) (polyHis-PEG) diblock copolymers and respond to acidity through the ionization of the imidazole group on the histidine. Upon protonation, the imidazole groups become hydrophilic and cause the disintegration of the micellar structure [45]. For example, NPs made of polyHis-PEG and poly(L-lactic acid)-PEG (PLLA-PEG) showed differential drug release kinetics at several pH values. Using Dox as the model drug, over a 10 hr period the PHSM with 20 wt% PLLA-PEG released 25% of Dox at pH=7.4, 39% at pH=6.5, 77% at pH=6.0, and 82% at pH=5.5. The release profile of the PHSM can be tuned by changing the weight ratio between the polyHis-PEG and PLLA-PEG in the formulation. These PHSM have shown favorable *in vitro* therapeutic efficacy against MDR cells. In a cytotoxicity study using folate as the targeting ligand and ovarian A2780 Dox-resistant carcinoma cells as the MDR cell model, the Dox-loaded PHSM showed 82% of growth inhibition in pH 7.4 media whereas both free Dox and Dox-loaded pH-insensitive micelles showed negligible cytotoxicity. In addition, the cytotoxicity of the PHSM was decreased in more acidic growth media, indicating premature drug release before cellular uptake [46]. The enhanced growth inhibition by PHSM is correlated to the accelerated intracellular drug release, which caused an instantaneous increase in intracellular drug concentration that overwhelmed P-gp drug efflux.

Instead of particle destabilization, some pH-sensitive NPs release their drug payloads more rapidly in acidic pH via physical expansions. Examples of these expansile NPs include polyHis-containing nanogel [47] and hydrogel NPs that contain acid-labile leaving groups [48]. A key feature underlying both of these NP systems is the increase in hydrophilicity under mildly acid condition and the subsequent water uptake. In the case of nanogel, which consists of a hydrophobic polyHis core and two hydrophilic layers of PEG and albumin, respectively, ionization of the imadizole groups in polyHis reversibly swells the particles. The nanogel exhibited repeated cycles of expansion and contraction (55 nm to 355 nm in diameter) in alternating pH values with corresponding changes in drug release rate (~4 fold increase from pH=7.4 to pH=6.4). In contrast, the size increase of the hydrogel NPs is irreversible as the monomers forming the particles permanently lose their hydrophobic protecting groups at low pH. The deprotection process exposes two additional hydroxyl groups on each monomer, significantly raising its hydrophilicity. As a result, a change of pH from 7.4 to 5 increased the particle volume by 350-fold and drasti-

cally sped up its drug release kinetics. *In vivo* studies with paclitaxel-loaded hydrogel NPs demonstrated higher efficacy in lung cancer treatment compared to its non-expansile counterparts [48].

ENDOCYTIC UPTAKE OF DRUGS TO BYPASS MDR

Not only can NPs combat MDR through enhancing drug accumulation at tumor sites, they can also directly tackle drug resistance mechanisms that are due to multidrug transporters such as P-gp. In MDR cells, transmembrane drug-efflux pumps actively expel intracellular drugs to the extracellular space, creating a huge barrier of entry on the cellular level. Free drug molecules that enter cells through either passive diffusion or membrane translocators are rapidly vacuumed out of the cells before they can take effect. In contrast, therapeutic NPs can partially bypass the efflux pumps as they are internalized through endocytosis [49, 50]. Once being engulfed by the plasma membrane, NPs are transported by endosomal vesicles before unloading their drug payloads. Thus drug molecules are released farther away from the membrane-bound drug efflux pumps and therefore are more likely to reach and interact with their targets. Numerous studies have shown that NP encapsulation results in better drug effectiveness against resistant cell lines. For example, the liposomal formulation of digoxin showed higher intracellular uptake and enhanced ability to overcome MDR compared to free digoxin [51]. In another study, it was demonstrated that Dox encapsulated in polyalkylcyanoacrylate NPs was more cytotoxic to P388 resistant cells than free Dox. The IC50 of Dox-loaded NPs was 800 ng/mLwhile of free Dox was 20000 ng/mL. Similar efficacy enhancement has been observed for many other therapeutic NP platforms [51-54], suggesting that endocytic transport is a viable strategy to circumvent P-gp mediated MDR effects.

CO-DELIVERY OF CHEMO-SENSITIZING AGENTS

Many chemo-sensitizing agents have been co-encapsulated with anticancer drugs in NPs to further suppress MDR effect. These chemo-sensitizing agents are typically P-gp modulators such as cyclosporin and verapamil that reduce P-gp activity or expression. Safety concerns have limited the clinical use of P-gp modulators because these transmembrane pumps are also present in healthy tissues such as liver, kidney, and blood-brain barrier, serving as a protective barrier against foreign molecules [55]. Targeted delivery of therapeutic NPs may offer a solution to minimize the undesirable side effects of P-gp modulators by protecting them from non-cancerous cells.

Incorporating P-gp modulators to drug-loaded NPs has shown great promises in reducing drug efflux and reverting MDR phenotype. For example, Soma *et al.* have encapsulated cycloporin A (CyA), a compound that binds directly to P-gp and inhibits its activity, together with Dox in polyalkylcyanoacrylate NPs [15]. In preparing the combinatorial NPs, CyA and Dox were mixed with the isobutylcyanoacrylate monomers during the emulsion polymerization process. As the NPs were formed, CyA was absorbed onto the particle surface whereas Dox was embedded in the polymeric core. Activities of both CyA and Dox were preserved as the combinatorial NPs showed higher growth inhibition on P388 Dox-resistant cells as compared to the NPs loaded with Dox alone. The measured IC50 value of CyA-Dox-loaded NPs and Dox-loaded NPs was 450 ng/mL and 800 ng/mL, respectively. In addition, the study also demonstrated that NPs enabled the synergistic effect between CyA and Dox because free CyA in solution failed to improve the growth inhibition ability of Dox-loaded NPs.

Curcumin is another P-gp modulator that has been co-encapsulated in NPs with anticancer drugs [13]. Curcumin is a naturally occurring compound that downregulates P-gp expression and facilitates apoptotic signaling. Because of its hydrophobic characteristic, curcumin was readily encapsulated in oil-in-water nanoemulsions along with paclitaxel. Results from western blotting revealed that the P-gp expression in paclitaxel-resistant SKOV3 human ovarian adenocarcinoma cells was significantly reduced after curcumin nanoemulsion treatment. The downregulation of P-gp contributes to the higher cytotoxicity of the combinatiorial nanoemulsions, whose IC50 is 1.8 fold less than that of the nanoemulsions loaded with paclitaxel alone. Further study found that the combinatorial treatment inhibited NFkB, a transcription factor that induces the expression of anti-apoptotic proteins and is closely related to paclitaxel resistance [56]. These findings suggest that therapeutic NPs co-delivering chemo-sensitizing agents and anticancer drugs can effectively suppress cancer drug resistance by resensitizing the MDR cells to chemotherapeutic treatment.

Not only can modulators of P-gp or other multidrug transporters be co-delivered with anticancer drugs by therapeutic NPs, compounds that modify other cellular activities can also be co-delivered to restore the mutated biochemical processes in drug resistant cells. For example, ceramide is a secondary messenger in the signaling cascade of the apoptotic pathway and it has been loaded into therapeutic NPs to overcome MDR [57]. Environmental stress such as cytotoxic agents elevates the intracellular ceramide level, which then promotes cell death by apoptosis. Some MDR tumors, however, exhibit a high level of glucosylceramide synthase that metabolizes ceramide to its inactive glycosylated form. These tumors are thus less likely to initiate the apoptotic process when treated with chemotherapeutic agents. In an attempt to revive the dysfunctional apoptotic signaling, ceramide was co-encapsulated with paclitaxel in poly(ethylene oxide)-poly(epsilon caprolactone) (PEO-PCL) NPs. It was found that the combinatorial NPs showed 100% cellular growth inhibition against SKOV3 paclitaxel-resistant cells at the IC50 dose of paclitaxel. This higher cytotoxicity translated to a 100-fold increase in chemosensitization of the co-delivery therapeutic NPs. Apoptotic activity analysis and western blotting study further revealed that the enhanced therapeutic efficacy was indeed due to the restoration of the defunct apoptotic pathway, indicating that drug resistance was reversed.

In addition to the use of MDR modulators as a combinatorial agent, co-delivering multiple chemotherapeutic drugs also opens up countless combination schemes to target the multi-faceted nature of MDR. Many anticancer compounds, both hydrophobic and hydrophilic [58], have been successfully encapsulated into the same NPs to treat cancers. It is not far-fetched to speculate that the numerous clinical combination chemotherapy regimens could be made more effective through NP-based co-administration. The therapeutic NPs can not only shield the combinatorial drugs from healthy tissues and prevent complex side effects but also uniformly deliver them to the target cells, that otherwise have different transport dynamics. The simultaneous uptake of multiple chemotherapeutic drugs will significantly reduce the chance of cancer cells acquiring immunity.

CONCLUSIONS

In conclusions, cancer drug resistance remains a major obstacle in cancer treatment. The development and manifestation of drug resistance are correlated with inefficient drug delivery to tumor sites. Therapeutic NPs are emerging as a safer and more effective drug delivery option as compared to their small molecule chemotherapy counterparts. They have shown numerous favorable features including long systemic circulation lifetime, targeting ability, stimuli-responsive drug release kinetics, cellular internalization through endocytosis and co-delivering multiple therapeutic agents. These desirable features make therapeutic NPs highly promising in combating cancer drug resistance.

OUTLOOK

Despite the great progresses having been made on therapeutic NPs, we call attention to a few key unmet challenges on developing therapeutic NPs to combat cancer chemoresistance. First, precisely co-delivering multiple drugs with different solubility remains challenging. Even though both hydrophobic and hydrophilic drugs have

been loaded in various NP systems, molar ratios between them are often incomparable and difficult to control, thereby compromising their combinatorial effects. Precise control over combinatorial drug loading would enable dosage optimization and maximize the synergism among the encapsulated compounds. Secondly, escape of NPs from the degradative endo-lysosomal vesicles to the cytoplasm is still challenging for many types of therapeutic NPs. Upon endocytosis, drugs in the NPs are subject to metabolism by the enzymatic and acidic environment in the endosomal and lysosomal compartments. A timely endosomal escape would greatly increase the therapeutic efficacy of the therapeutic NPs. Lastly, the transport property of therapeutic NPs through solid tumors is poorly understood. It is crucial to ensure that NPs are able to transport from tumor vasculatures to tumor beds and penetrate through tumor periphery to deep tumor tissues in order to effectively eliminate all cancer cells. Overall, we believe that continuous research efforts on NP-based therapeutics will provide solutions to effective cancer treatment that scientists and physicians have been searching for decades.

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