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Co-delivery of dual-drugs with nanoparticle to overcome multidrug resistance

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Review

Abstract: Multidrug resistance (MDR) is becoming a significant obstacle for chemotherapeutic treatment in the battle against malignant cancers. Because of the emergence of MDR, higher doses of chemotherapeutic drugs are needed which eventually leads to intolerable toxicity and the death of patients. Different from the normal drug resistance that only resists to the previously administrated drugs, multidrug resistance diminishes the efficacies of a wide range of chemotherapeutic drugs, causing the decrease of intracellular drug concentration and the failure of killing enough cancer cells. Co-administration of multiple chemotherapeutic drugs cannot overcome MDR due to the different pharmacokinetic properties of combined drugs and only brings limited clinical advantage for the patients. Recent progress in nanomedicine and nanotechnology has enabled biomedical scientists to deliver multiple drugs of similar or different acting mechanisms into cancer cells with a predefined releasing profile. Thus, combining nanotechnology and co-delivery technique has the great potential to selectively deliver multiple drugs to overcome MDR and benefit cancer patients.

Key words: Nanomedicine, multidrug resistance, co-delivery, drug delivery, self-assembly, gene therapy.

Introduction

Chemotherapy still plays a vital role in the treatment of different types of cancer. However, the effectiveness of chemotherapy is severely hindered by the emergence of multidrug resistance (MDR), which causes over 90% che-motherapeutic failures of cancer treatments (1). Although several different cellular mutations can provoke MDR, two of them are under extensive research interests, namely increase of cell surface mediated drug efflux pumps and the up-regulation of anti-apoptotic pathways (2). In the first scenario, MDR comes from intrinsic high expression of ATP-binding cassette (ABC) transporter proteins inclu-ding P-glycoprotein (P-gp), multidrug resistance proteins (MRP) and breast cancer resistance protein (BCRP). For example, P-gp can be found overexpressed in lots of MDR cancer cells such as liver, pancreatic and ovarian cells (3). They can bind with a variety of structurally different che-mical compounds and pump them out of the cytoplasm of cancer

cells, decreasing the cellular concentration of active drugs and finally leading to low therapeutic outco-me (4-8). For example, human KB adenocarcinoma cells line KB-CP20 whose multidrug resistance was acquired by treatment with cisplatin and the associated overexpres-sion of MDR1 gene, can also be resistant to many other cytotoxic agents such as alkylating agents, methotrexate (MTX) and platinum analogues (9). For the other case, significant reduction of cellular response to apoptosis si-gnals is often observed and can be attributed to the dere-gulation in the apoptotic-pathways such as bcl-2 or NF-κB, enabling cancer cells to tolerate drug-induced injuries and acquire MDR in the end (10,11).

To address the severe problems of MDR, co-admi-nistration of different chemotherapeutic agents was exa-mined in clinic. However, only very limited efficacy was observed possibly due to the different pharmacokinetic properties of multiple drugs (12). With the advancement of nanomedicine, gene engineering (13-17) and big data (18-22), it is now possible to improve the anticancer in-dexes with nanoparticle drug delivery system, which can reduce the toxicity of chemotherapeutic agents with hi-gher selectivity and is a promising solution to overcome the MDR problems in cancer chemotherapy (23-26). By carefully designing the delivery system, nanoparticles are able to protect the payloads (chemotherapeutic drugs or MDR sensitizer) during the circulation and release loaded drugs in a predefined pharmacokinetic way (27-31). The unique size of nanoparticles can also grant themselves with the "enhanced permeability and retention effect" (EPR) which allows nanosized particles to be specifical-

Table 1. Summary of Listed Nanoparticles with Dual Drug Delivery to Overcome Multidrug Resistance.

Materials	Agent 1	Agent 2	Cancer cell lines	Ref
Poly(ε-caprolactone) and poly(ethyl ethylene phosphate)	Doxorubicin	NA	MCF-7/ADR	(38)
Poly(lactic-co-glycolic acid) and polyethylene glycol	Vincristine	NA	MCF-7/ADR	(42)
Pluronic-P105	Doxorubicin	Paclitaxel	MCF-7/ADR	(46)
Poly(ethylene oxide)-poly(propylene oxide)-poly(ε-caprolactone)	Docetaxel	Chloroquine	MCF-7 and MCF-7/ADR	(47)
VE and tocopherol poly(ethylene glycol)succinate	Paclitaxel	5-Fluorouracil	KB-8-5 and KB-3-1	(48)
Poly (lactic-co-glycolic acid)	Docetaxel	TPGS	HeLa	(50)
EPC, DOTAP, cholesterol and PEG2KPE	Paclitaxel	Tariquidar	SKOV-3 and SKOV-3TR	(51)
CEA and AHM	Doxorubicin	Verapamil	NCI/ADR-RES	(53)
Chitosan	Doxorubicin	Pyrrolidinedithiocarbamate	HepG-2	(54)
TPGS2000 and PEG2000-DSPE	Doxorubicin	Curcumin	MCF7/ADR	(55)
Poly(D,L-lactide-co-glycolide)	Doxorubicin	Curcumin	K562	(56)
1-Palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine	Doxorubicin	Ceramide	P388/ADR	(57)
Precirol ATO 5,Squalene, SPC, Tween-80 and DOTAP	Doxorubicin/Paclitaxel	siRNA targeting MRP1 and BCL2	A549	(62)
PAMAM and PEG-2K-DOPE	Doxorubicin	siRNA targeting GFP	A549 cells and C166-GFP	(63)

ly targeting and accumulating in the tumor cells due to the more leaky nature of tumor vasculature (32-35). Therefore, combination of nano drug delivery systems and co-delivery of multiple anticancer drugs holds the great potential to surmount the MDR of cancer cells (32). In this review, we will discuss the recent progress of using nanoparticle to co-deliver multiple pharmaceutical agents to battle against MDR. The delivery of single and multiple traditional chemotherapeutic drugs by nanoparticle with increased anti-MDR effects will be reviewed first. The combination of chemotherapeutic drugs with MDR modulator, sensitive agents as well as therapeutic nucleotides such as regulating siRNA in nanoparticles will also be covered.

With the help of EPR effect of nanoparticles, sufficiently high intracellular level of cytotoxic chemicals can be achieved while using an optimized nanoparticle as delivery system, which may help overcome the MDR of cancer cells (10,11,36,37). Wang et al managed to deliver doxorubicin (DOX) with disulfiide-bridged diblock copolymer PCL-SS-PEEP (38). Because of the poly(ethyl ethylene phosphate) (PEEP) constructed shell, the synthesized nanoparticleffi has high affinity to cancer cells and is more e ciently internalized by tumor cells, assisting the escape of active drugs from the pump-off by P-gp and leading to high levels of cellular drug accumulation (Fig.1). As a result, DOX encapsulated PCL-SS-PEEP nanoparticle shows a rapid release of the free DOX in MDR cancer cells. On the other hand, MDR in cancer cells is often associated with an elevation in the concentration of reductive glutathione (GSH), which makes the disulfide linkage vulnerable and can selectively break disulfide linkage within the PCL-SS-PEEP system in MDR cells, releasing the active DOX inside tumor cells. In vitro study shows the IC50 value of DOX-loaded PCL-SS-PEEP was only 32% of DOX-loaded PCL-b-PEEP nanoparticles (without disulfide), proving the enhanced selectivity of disulfide-based nanoparticle. In the meantime, a significant decrease of the IC50 value of DOX-loaded PCL-SS-PEEP in multi-drug resistant MCF-7/ADR cell lines was also observed (~2μg/ml) as compared with free DOX treatment (~20ug/ml).

Besides the inherent EPR effect, nanoparticles decorated with selective ligands such as folate or targeting pep-

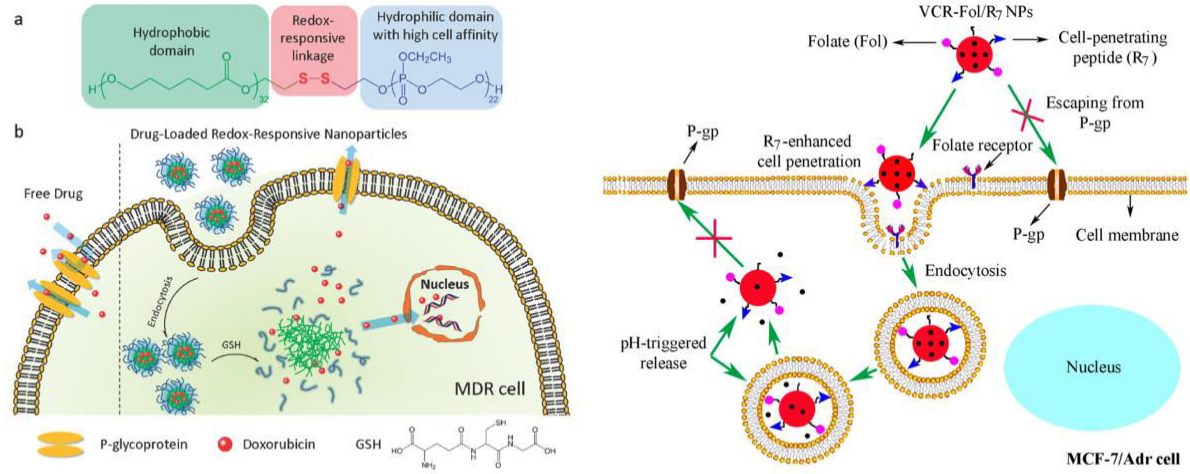

Figure 1. left). Chemical structure of PCL-SS-PEEP block copolymer and schematic illustration of redox-responsive nanoparticles; right).Sche-matic representation of VCR-Fol/R7 NPs. Reprinted with permission from (38) & (42). Copyright (2016) American Chemical Society.

tides can further enhance the selectivity and the absorption of active therapeutic agents into tumor cells, leading to the increase of the cellular concentration of active drugs (39-41). Demonstrated by Wang et al (42), a multifunctional poly(lactic-co-glycolic acid) (PLGA) and polyethylene glycol (PEG) nanoparticle with folate and cell penetrating peptide (CPP) was prepared for delivering vincristine sulfate (VCR) to tumor and overcoming MDR (Fig.1). A unique feature here is the dual decoration of nanoparticles with targeting ligand folate and cell penetrating peptide R7 for the increase of accumulation of VCR. The overex-pression of folate receptors on human breast carcinoma cells together with strong intracellular penetration effect from arginine-rich CPP R7 allow the synthesized nano-particles to accumulate VCR in resistant tumorffl cells by escaping P-glycoprotein mediated drug e ux. In vitro studies shows that VCR-Fol/R7 nanoparticles exhibited much higher cytoxicity (IC50: 27.1 ± 1.47 μg/mL) than that of free VCR (IC50: 479.80 ± 8.62 μg/mL) in MDR MCF-7/ ADR cells. In vivo study also suggests the strongest anti-tumor efficacy of the dual decorated nanoparticles while evaluated in nude mice bearing MCF-7/ADR tumors com-pared with either single VCR or mono-decorated nanopar-ticle, indicating the promising future of VCR-Fol/R7 na-noparticles toffiovercome multidrug resistance and improve therapeutic e cacy.

Co-delivery of dual chemotherapeutic drugs

Co-delivery of anticancer drugs with similar or diffe-rent mechanisms of action has been found to be a pro-mising strategy to overcome undesirable toxicity and other side effects as well as to improve therapeutic index (43-45). Chen et al successfully constructed a surfactant nanoparticle with Pluronic P105-DOX conjugate contai-ning a hydrophobic core (46), which can be used to entrap another chemotherapeutic drug paclitaxel (PTX). DOX is generally considered a hydrophilic anticancer drug binding with DNA by intercalation while PTX is highly hydrophobic and inhibits microtubules disassembly to induce apoptosis. Patients with metastatic breast cancers benefits from the co-administration of DOX and PTX. Therefore, the dual drug loaded micelles (PF-DOX-PTX) offer great advantages in terms of achieving synergistic effect of these two drug substances. Study results showed that a sustained release profile can be achieved for both DOX and PTX in vitro with effectively accumulation of both drugs in MDR cancer cells MCF-7/ADR. Both DOX and PTX release rates are increased in pH = 5.0 buffer which mimics the acidic microenvironment of lysosome compared with normal pH = 7.4 PBS buffer. Furthermore, in vitro cytotoxicity, cell apoptosis and cell cycle arrest studies indicated that PF-DOX-PTX had better antitumor efficacy in MDR cancer cells compared to those of single-drug loaded micelles. More importantly, a much stronger antitumor efficacy in MCF-7/ADR tumor-bearing mice was observed in PF-DOX-PTX group than that of com-bined administration of free DOX and PTX. For example, PF-DOX-PTX shows IC50 values as 0.0017 and 0.328 μg/ mL while co-administration of DOX and PTX only has IC50 of 0.014 and 15.011 μg/mL in MCF-7 and MCF-7/ ADR cell lines respectively. This example proves the su-perior efficiency of co-delivery chemotherapeutics with nanoparticles over the conventional co-administration.

Chloroquine (CQ) can be selectively accumulated in lysosomes and execute its anti-tumor function through inhibition of autophagy. Therefore, combining CQ with certain anticancer drugs is a good choice to inhibit auto-phagy-dependent resistance for chemotherapy and enhance the anticancer effects. Shi et al co-delivered anticancer drug docetaxel (DTX) and CQ in a complex micelle com-posed of PEO-PPO-PCL (poly(ethylene oxide)-poly(pro-pylene oxide)-poly(ε-caprolactone)) and TPGS (D-α-to-cophery1 poly(ethyleneglycol)) for enhancing anticancer effects (47). The dual-drug loaded nanoparticle possessed sphere shape micelle structures with size of 31.2 nm and 115.4 nm depending on different length of PCL. In vitro study of cellular uptake demonstrates that the micelles can effectively accumulate in MDR cancer cells MCF-7/ ADR. Furthermore, the co-delivery micelles have higher therapeutic effects against MCF-7 and MCF-7/ADR cells than either free drug or individually DTX-loaded micelles. The IC50 values of DTX/CQ-loaded PEO68-PPO34-PCL18/ TPGS and PEO68-PPO34-PCL36/TPGS micelles are 134.16 and 194.74 folds smaller than that of free DTX after 48 h treatment with MCF-7/ADR cells respectively, showing the co-delivery of DTX and CQ with nanoparticle can provide a promising combined therapeutic strategy for en-hanced antitumor therapy.

The co-administration of paclitaxel (PTX) and 5-fluo-roucacil (5-FU) has been tested to show promising ef-ficiency in MDR cancer therapy such as on gastric or breast cancer patients. However, it is very challengingffto control the molar ratio of the two drugs and tune the di e-rent pharmacokinetic and bioavailability properties with simple co-administration. To address this problem, a core-matched nanoemulsions (NE) functionalized by vitamin E (VE) and tocopherol poly(ethylene glycol)succinate (TPGS) was developed by Ma et al to co-deliver hydro-phobic and hydrophilic drugs (PTX andff 5-FU) at the same time in order to achieve synergistic e ects and overcome PTX resistance in a MDR human epidermal carcinoma

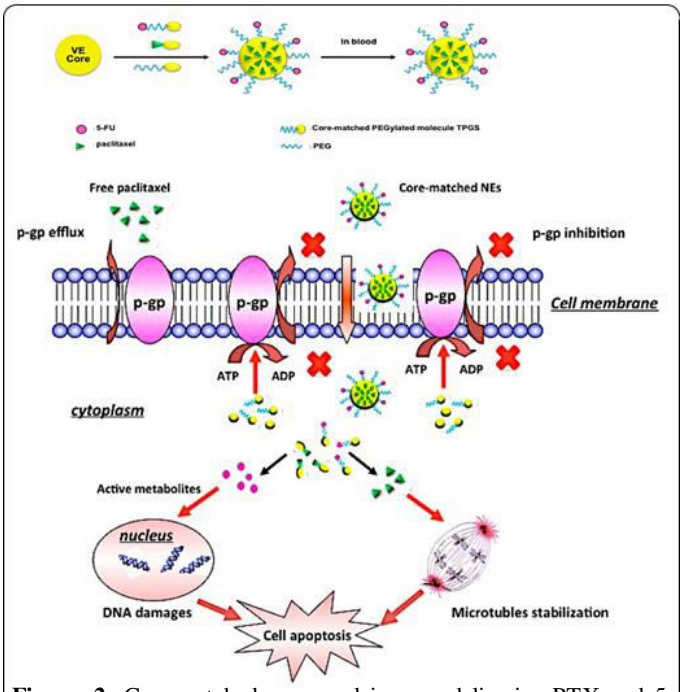

Figure 2. Core-matched nanoemulsions co-delivering PTX and 5-FU to achieve reversal of MDR. Reprinted with permission from (48). Copyright (2016) American Chemical Society.

cell line (48). The nanoemulsion was obtained by mixing PTX-VE, 5-FU-TPGS, TPGS and VE in chloroform fol-lowed by evaporation and remixing in distilled water. The dual-drug loaded NE exhibited concentration and time-de-pendent cytotoxicity against PTX-sensitive KB-3-1 cells and PTX-resistant KB-8-5 cells (Fig.2). Interestingly, the IC50 of PTX-VE-5-FU-TPGS nanoemulsion was 0.54 μM in KB-8-5 cells and 1.38 μM in KB-3-1 cells, showing a resistant index 0.39. While without the co-delivery strate-gy, the resistant index of PTX increases to 2.96, showing that the synergism of PTX and 5-FU could reverse MDR through co-delivery in a single nanoparticle for cancer therapy.

Co-delivery of Chemotherapeutics with MDR Inhibi-tor

Therapeutic nanoparticles containing the combination of cytotoxic drugs and efflux pump (e.g, P-gp) inhibitors, such as cyclosporine, verapamil and tariquidar, hold the potential to suppress MDR effect while still maintaining effective cytotoxicity (49). Such co-delivery strategy can also address the poor pharmacokinetic properties and high systemic toxicities often associated with MDR inhibitors. Zhu et al used the poly (lactic-co-glycolic acid) (PLGA) nanoparticle for co-delivery of docetaxel(DTX) with to-copherol poly(ethylene glycol)succinate (TPGS) (50). The co-delivered TPGS can serve as an active matrix compo-nent to inhibit P-gp ATPase and drug effllux for overco-ming MDR. Four different DTX-loaded PLGA NPs with 0, 10, 20 and 40% of TPGS were prepared and found of size ranged 100 to 120 nm and encapsulation efficiency between 85 to 95% at drug loading level around 10%. The IC50 values of the free DTX and the aforementioned NPs of 0, 10, 20% TPGS were 2.619 and 0.474, 0.040, 0.009 μg/mL respectively after incubated in HeLa cells for 48 hours, showing great improvement in cell cytotoxicity. The increase of cell cytotoxicity can be attributed to the inhibition of P-gp ATPase from TPGS, resulting in higher cellular concentration of DTX and better therapeutic effi-cacy both in vitro and in vivo.

Tariquidar is another potent and specific P-gp inhibitor and can sensitize the resistant cell lines with great promise to reverse the MDR caused by the P-gp overexpression. One of the biggest concerns for the combination use of tariquidar and chemotherapeutics is the non-specific bin-ding of tariquidar to the P-gp in normal tissues such as blood brain barrier and gastrointestinal track. Obviously, higher selectivity provided by nanoparticle co-delivery system can help address the non-specificity issue. Patel et al co-delivered tariquidar and paclitaxel (PTX) with long-circulating liposomes to reverse the MDR (51). The liposome is composed of L-α-phosphatidylcholine (EPC), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), cholesterol (CHOL) and polyethylene glycol 2000-phos-phatidylethanolamine (PEG2KPE). Tariquidar and pacli-taxel-loaded co-delivery liposomes showed significant re-sensitization of chemo drugs to the resistant cancer cells. For example, the IC50 values for PTX were 27.11 nM and 2743 nM in SKOV-3 and MDR SKOV-3TR cells when treated with paclitaxel alone. The value can dramatical-ly decrease to 17.68 nM and 34 nM respectively when treated with synthesized dual-drug delivered liposomes, demonstrating the reversal of the MDR by the co-delive-ring tariquidar with active cytotoxic drugs.

Verapamil (Vera) is a non-dihydropyridine calcium channel blocker, normally used for the treatment of hy-pertension, angina pectoris and cardiac arrhythmia. It was also discovered that Vera can block the pathway of a drug effflux pump such as P-gp, making it a great candidate for co-delivery strategy (52,53). Qin et al engineered a hy-drogel nanoparticle composed of co-polymerized acryla-mide (AAm), 2-carboxyethyl acrylate (CEA) and 3-(acry-loyloxy)-2-hydroxypropyl methacrylate (AHM) (54). The resulting nanoparticle was then to serve as a carrier to co-deliver DOX and Vera, aiming at alleviating tumor MDR. One unique feature here is either Vera or Dox is post-loaded into nanoparticles, which gives more freedom to adjust the relative ratios of Vera and DOX. DOX resis-tant NCI/ADR-RES cells were used to study the in vitro cytotoxicity of the synthesized drug-loaded nanoparticles. While the IC50 values of DOX and DOX only nanopar-ticles were >20 and 19 μM, co-delivery of DOX nanopar-ticle with Vera gives IC50 value of 4.5 μM compared with that of 10 μM when co-administrated with DOX and Vera. More importantly, co-delivery of DOX nanoparticle and Vera nanoparticle shows the strongest antitumor efficien-cy with IC50 of only 2.5 μM, suggesting a promising route for overcoming MDR.

Co-delivery of Chemotherapeutics with Sensitizer

In response to MDR caused by the alterations of the apoptosis pathways, sensitizer compounds that can repair the dysfunctional apoptotic signaling are being delivered with chemotherapeutic drugs within nanoparticles. One example was demonstrated by Fan et al to use folate-chi-tosan (FA-CS) nanoparticle for the co-delivery of pyrro-lidinedithiocarbamate (PDTC) and doxorubicin (DOX) (55). PDTC is a NF-κB inhibitor and often used as a sen-sitizer of the anticancer drug. NF-κB is widely used as a regulator of genes to control cell proliferation and cell survival. Therefore, resensitizing NF-κB pathway could enhance the sensitivity of tumor cells to apoptosis and help reduce the related MDR. Compared with pure DOX and micelle without either FA or PDTC, the FA-CS na-noparticle with both DOX and PDTC shows lowest IC50 in multidrug resistant HepG-2 liver cancer cells as 0.028 mg/L. Without co-delivery with PDTC, the IC50 value in the same cell lines increases to 0.059 mg/L, showing that co-delivery with sensitizer can restore and even enhance the cytotoxicity of delivered chemotherapeutics. Through folate mediation, FA-CS/DOX- PDTC nanoparticle also has more cellular uptake of DOX than pure DOX and FA-CS/DOX micelle in HepG-2 cells.

Curcumin (CUR) is a polyphenol with several biologi-cal functions such as anticancer activity. CUR can also act as a mediator to down-regulate both PI3K/AKT and NF-κB pathways. PI3K/AKT pathway is found to be overac-tive in many MDR cancer cells, allowing reduced apopto-sis and increased proliferation. Co-delivery with curcumin is then promising for chemotherapeutic drugs to escapes from MDR. Wang et al designed polymeric micelles with TPGS2000 (Di-tocopherol polyethylene glycol 2000 succinate) and PEG2000-DSPE (1,2-distearoyl-sn-gly-cero-3-phosphoethanolamine-N-[methoxy-(polyethyle-ne glycol)-2000) to co-deliver DOX and CUR with high encapsulation effiiciency and stability (56). Co-delivery

with CUR increases the cellular uptake and improves an-ticancer effiicacy of DOX in multidrug resistant MCF7/ ADR cells. Meanwhile, co-delivery of DOX and CUR in nanoparticle micelle signifiicantly enhances cellular apop-tosis in vitro, which induced 74.9% of MCF7/ADR cell apoptosis compared with only 4.1% for DOX-only treat-ment. Remarkable loss of tumor weight was also observed in vivo, suggesting the synergistic effects of co-delivery of DOX and CUR. Similarly, Misra et al investigated the co-delivery of DOX and CUR into chronic myeloid leukemia K562 cells with poly(*D,L*-lactide-*co*-glycolide) (PLGA) nanoparticles (57). With the co-delivery of CUR, the expression of MDR1 and BCL-2 at the mRNA level in K562 cells were dramatically decreased from 43.4 to 3.5 fold and 6.50 to 1.07 fold respectively. Attractively, DOX-CUR nanoparticle also shows strongest in vitro cytotoxi-city in chronic myeloid leukemia K562 cells compared with DOX-CUR solution. The IC50 value of DOX-CUR nanoparticle was 0.1±0.93 μg/ml while that of DOX-CUR solution was 6.7±0.91 μg/ml. Both of these two examples demonstrate that the co-delivery of DOX with CUR has signifiicant synergistic effects to bring success against MDR in cancer therapy.

Lipid C6 ceramide serves as a key messenger in programmed cell death and can be used to decrease the apoptosis threshold in tumor cells. Wang et al developed a nanoparticle micelle formulation to deliver DOX with ceramide into multidrug resistant leukemia cells P388/ ADR (58). The lipid nano micelle was composed of 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine (PazPC), DOX, ceramide and PEG-2000. The study shows DOX-ceramide micelle has IC50 value of 226.78 nM against MDR P388/ADR cells compared with 523.38 nM from the no-ceramide DOX micelle, showing the ef-fectiveness of delivering ceramide to increase the efficacy of DOX against drug resistant leukemia cells.

Co-delivery of Chemotherapeutics with siRNA

The advancement of gene engineering especially the progress in small interfering RNA(siRNA) technology has provided cancer treatment with promise highly speci-fic therapeutic weapons (59,60). siRNA works through in-terfering with the expression of specific genes with com-plementary nucleotide sequences, resulting in no transla-tion (2). While direct of injection of the RNA molecules is not effective due to the rapid degradation of the molecule in serum, nanoparticles can serve to entrap the siRNA as cargo and protect them from the outside environment, lea-ding to the potential success of overcoming MDR (61,62). Thus, co-delivery of MDR-targeted siRNA such as tar-geting MRP1 or BCL2 with cytotoxic drugs is attracting more and more interests from biomedical scientists.

Taratula et al developed a multifunctional nanostruc-tured lipid carrier (NPL) to co-deliver anticancer drugs and multiple siRNA (BCL2 and MRP1) to enhance antitumor activity and suppress multidrug resistance (63). A key fea-ture here is that the synthesized nanoparticle contains dual siRNA silencing both BCL2 and MRP1 besides the en-capsulated chemotherapeutic drugs (doxorubicin or pacli-taxel). Meanwhile the surface is also decorated with a mo-dified synthetic analog of luteinizing hormone-releasing hormone (LHRH) as a targeting agent towards lung cancer cells. In vitro test shows nanoparticle with both PTX, siRNA and LHRH has best anticancer effects with IC50 value less than 1 μg/ml while the nanoparticle with only PTX and LHRH shows IC50 value around 10 μg/ml human lung cancer cells A549. RT-PCR reveals that both of the loaded siRNA (targeted to MRP1 and BCL2 mRNAs) successful-ly suppressed targeted mRNA isolated from A549 human lung cancer cells after being released from the delivery nanoparticle. In vivo study of lung tumor models shows the fully equipped LHRH-NLC-TAX-siRNAs (MRP1 and BCL2) can shrink the tumor size from 45 mm3 to 2.6 ± 3.0 mm3 while the one without targeting siRNA can only make it to 20.8 ± 4.4 mm3 after 4 weeks treatment.

Dendrimers are repetitively branched molecules with highly symmetric spherical shape. Cationic dendrimers have positively charged surface and are often used to bind and delivery negatively charged biomolecules through the electrostatic interaction. Biswas et al used poly(ethylene glycol)-dioleoylphosphatidyl ethanolamine (PEG-DOPE) to construct nanoparticle micelles for effective delivery of hydrophobic anticancer drugs (64). At the meanwhile, a G(4)-Polyamidoamine (PAMAM) cationic dendrimer was synthesized as a cationic carrier of MDR targeting siRNA and conjugated with PEG-DOPE micelle (G(4)-D-PEG-DOPE), which can form nanoparticle with diame-ter around 60.4 nm. Two micelle systems, G(4)-D-PEG-DOPE (MD) and G(4)-D-PEG-DOPE/PEG-DOPE(1:1, MDM), were both evaluated for drug delivery efficiency and cytotoxicity. After self-assembly, the resulting nano micelles can be used to co-deliver the binded siRNA and the encapsulated anticancer drug (DOX). GFP-specifiic siRNA was used as a model nucleotide and in vitro gene silencing shows the silencing effiicacy of both MD and MDM were signifiicantly higher (22%, 18% respectively) in the siRNA-PEG-DOPE-Dendrimer complex compared with G(4)-D (10%) only, showing the great potential of using this technique to circumvent the problem of MDR.

Conclusion

Combination of nano drug delivery systems and co-delivery of multiple drugs holds the great potential to surmount MDR in the treatment of cancers. Different strategies based on different combination of drugs can be realized by carefully selection of nano delivery system. While co-delivery of multiple chemotherapeutic drugs with similar or different mechanisms can be beneficial to overcome MDR, future development of co-delivery with nanoparticle is expected to be focused on resensitizing re-sistant cancer cells by either MDR inhibitor or through the modulation of siRNA. Furthermore, the development of combinational use of siRNA with chemotherapeutic drugs can help advance the treatment with precision medicine, allowing biomedical scientist and oncologists to achieve the ideal combination therapy effects.

References

1. Gao Z, Zhang L, and Sun Y. Nanotechnology applied to overcome tumor drug resistance. Journal of controlled release. 2012, 162: 45-55.

2. Hu C M, and Zhang L. Nanoparticle-based combination therapy toward overcoming drug resistance in cancer. Biochemical pharmacology. 2012, 83: 1104-1111.

3. Chen Y, Chen H, and Shi J. Inorganic nanoparticle-based drug co-delivery nanosystems to overcome the multidrug resistance of cancer

cells. Molecular pharmaceutics. 2014, 11: 2495-2510.

4. Li W, Rao D K, and Kaur P. Dual role of the metalloprotease FtsH in biogenesis of the DrrAB drug transporter. Journal of biological che-mistry. 2013, 288: 11854-11864.

5. Li W, Sharma M, and Kaur P. The DrrAB efflux system of Strep-tomyces peucetius is a multidrug transporter of broad substrate specifi-city. Journal of biological chemistry. 2014, 289: 12633-12646.

6. Pradhan P, Li W, and Kaur P. Translational coupling controls ex-pression and function of the DrrAB drug efflux pump. Journal of mole-cular biology. 2009, 385: 831-842.

7. Yang Z G, Meng H, Zhang X, Li X D, Lv W L, and Zhang Q. Effect of quercetin on the acyclovir intestinal absorption. Journal of Peking

University. Health sciences. 2004, 36: 309-312

8. Zhang H, Rahman S, Li W, Fu G, and Kaur P. Characterization of a novel domain 'GATE' in the ABC protein DrrA and its role in drug efflux by the DrrAB complex. Biochemical and biophysical research communications. 2015, 459: 148-153.

9. Shen D W, Goldenberg S, Pastan I, and Gottesman M M. De-creased accumulation of [14C]carboplatin in human cisplatin-resistant cells results from reduced energy-dependent uptake. Journal of Cellu-lar Physiology. 2000, 183: 108-116.

10. Parhi P, Mohanty C, and Sahoo S K. Nanotechnology-based com-binational drug delivery: an emerging approach for cancer therapy. Drug Discov Today. 2012, 17: 1044-1052.

11. Liu F, Kozlovskaya V, Zavgorodnya O, Martinez-Lopez C, Cat-ledge S, and Kharlampieva E. Encapsulation of anticancer drug by hy-drogen-bonded multilayers of tannic acid. Soft Matter. 2014, 10: 9237-9247.

12. Cao Y, Wu K, Yang Z, Sun J, Zhang X, and Zhang Q. The effect of three different P-glycoprotein inhibitors on the pharmacokinetic be-haviors of paclitaxel. Chinese Journal of Hospital Pharmacy. 2007, 27: 877-880

13. Hu J, and Gao Z. Data-based core genes screening for Hepatocellu-lar Carcinoma. Industrial Electronics and Applications (ICIEA), 2014 IEEE 9th Conference on. 2014: 1102-1107

14. Hu J, Gao Z, and Pan W. Multiangle Social Network Recommen-dation Algorithms and Similarity Network Evaluation. Journal of Ap-plied Mathematics. 2013, 2013

15. Sharma S, Shandilya R, Patnaik S, and Mahapatra A. Leading NoSQL models for handling Big Data: a brief review. International Journal of Business Information Systems, Inderscience. 2015, 18

16. Liu T, and Kirn D. Gene therapy progress and prospects cancer: oncolytic viruses. Gene therapy. 2008, 15: 877-884

17. Kershaw M H, Westwood J A, and Darcy P K. Gene-engineered T cells for cancer therapy. Nature Reviews Cancer. 2013, 13: 525-541

18. Sharma S, Tim U S, Wong J, Gadia S, and Sharma S. A brief review on leading big data models. Data Science Journal. 2014, 13: 138-157

19. Sharma S. Expanded cloud plumes hiding Big Data ecosystem. Fu-ture Generation Computer Systems. 2016, 59: 63-92

20. Sharma S, Tim U S, Gadia S, Wong J, Shandilya R, and Peddoju S

K. Classification and comparison of NoSQL big data models. Interna-tional Journal of Big Data Intelligence. 2015, 2: 201-221

21. Murdoch T B, and Detsky A S. The inevitable application of big data to health care. The Journal of American Medical Association. 2013, 309: 1351-1352

22. Marx V. Biology: The big challenges of big data. Nature. 2013, 498: 255-260

23. Creixell M, and Peppas N A. Co-delivery of siRNA and therapeutic agents using nanocarriers to overcome cancer resistance. Nano Today. 2012, 7: 367-379.

24. Wang H-p, Sun Y, Zhou Y, and You Q-d. Advances in privileged structure 4-quinolones as antitumor agents research. Chinese Journal of Medicinal Chemistry. 2012, 22: 59-67

25. Sun Y, Wang H, Zhou Y, and YOU Q-d. Recent advances in

non-campto-thecin DNA topoisomerase I inhibitors as anticancer drugs. Progress in Pharmaceutical Science. 2011, 35: 385-395

26. Kang C, Sun Y, Wang M, and Cheng X. Nanosized Camptothecin Conjugates for Single and Combined Drug Delivery. European Journal of BioMedical Research. 2016, 2: 8-14

27. Wang X, Huang X, Yang Z, Gallego-Perez D, Ma J, Zhao X, et al. Targeted delivery of tumor suppressor microRNA-1 by transferrin-conjugated lipopolyplex nanoparticles to patient-derived glioblastoma stem cells. Current pharmaceutical biotechnology. 2014, 15: 839-
846

28. Xie J, Teng L, Yang Z, Zhou C, Liu Y, Yung B C, et al. A polyethy-lenimine-linoleic acid conjugate for antisense oligonucleotide delivery. BioMed research international. 2013, 2013: 710502.

29. Yang X, Yang S, Chai H, Yang Z, Lee R J, Liao W, et al. A Novel
Isoquinoline Derivative Anticancer Agent and Its Targeted Delivery to Tumor Cells Using Transferrin-Conjugated Liposomes. PLoS One. 2015, 10: e0136649.

30. Yang Z, Chang L, Li W, and Xie J. Novel biomaterials and biotech-nology for nanomedicine. European Journal of BioMedical Research. 2015, 1: 1-2

31. Yang Z, Yu B, Zhu J, Huang X, Xie J, Xu S, et al. A microfluidic method to synthesize transferrin-lipid nanoparticles loaded with siRNA LOR-1284 for therapy of acute myeloid leukemia. Nanoscale. 2014, 6: 9742-9751.

32. Kim S H, Kaplan J A, Sun Y, Shieh A, Sun H L, Croce C M, et al. The Self-Assembly of Anticancer Camptothecin–Dipeptide Nanotubes: A Minimalistic and High Drug Loading Approach to Increased Efficacy. Chemistry-A European Journal. 2015, 21: 101-105

33. Xue B, Kozlovskaya V, Liu F, Chen J, Williams J F, Campos-Gomez J, et al. Intracellular Degradable Hydrogel Cubes and Spheres for Anti-Cancer Drug Delivery. Acs Applied Materials & Interfaces. 2015, 7: 13633-13644.

34. Liu F, Kozlovskaya V, Medipelli S, Xue B, Ahmad F, Saeed M, et al. Temperature-Sensitive Polymersomes for Controlled Delivery of Anticancer Drugs. Chemistry of Materials. 2015, 27: 7945-7956.

35. Liang X, Liu F, Kozlovskaya V, Palchak Z, and Kharlampieva E. Thermoresponsive Micelles from Double LCST-Poly(3-methyl-N-vinylcaprolactam) Block Copolymers for Cancer Therapy. Acs Macro Letters. 2015, 4: 308-311.

36. Xue B, Deng D W, Cao J, Liu F, Li X, Akers W, et al. Synthesis of
NAC capped near infrared-emitting CdTeS alloyed quantum dots and application for in vivo early tumor imaging. Dalton transactions. 2012, 41: 4935-4947.

37. Chen Z, Wu C, Yang Y, Shi J, Hu J, Yang Z, et al. Synthesis and drug delivery of mesoporous silica nanoparticles for cancer therapy. European Journal of BioMedical Research. 2015, 1: 30-36

38. Wang Y C, Wang F, Sun T M, and Wang J. Redox-responsive nanoparticles from the single disulfide bond-bridged block copolymer as drug carriers for overcoming multidrug resistance in cancer cells. Bioconjugate chemistry. 2011, 22: 1939-1945.

39. Zhu H Y, Liu F, Guo J, Xue J P, Qian Z Y, and Gu Y Q. Folate-mo-dified chitosan micelles with enhanced tumor targeting evaluated by near infrared imaging system. Carbohydrate Polymers. 2011, 86: 1118-1129.

40. Zhou Y, Xu X, Sun Y, Wang H, Sun H, and You Q. Synthesis, cytotoxicity and topoisomerase II inhibitory activity of lomefloxacin derivatives. Bioorganic & medicinal chemistry letters. 2013, 23: 2974-2978

41. Guo X K, Sun H P, Shen S, Sun Y, Xie F L, Tao L, et al. Synthesis and evaluation of gambogic acid derivatives as antitumor agents. Part III. Chemistry & biodiversity. 2013, 10: 73-85

42. Wang Y, Dou L, He H, Zhang Y, and Shen Q. Multifunctional Na-noparticles as Nanocarrier for Vincristine Sulfate Delivery To Overco-me Tumor Multidrug Resistance. Molecular Pharmaceutics. 2014, 11:

885-894.

43. Sun H, Zhu J, Chen Y, Sun Y, Zhi H, Li H, et al. Docking Study and Three-Dimensional Quantitative Structure-Activity Relationship (3D-QSAR) Analyses and Novel Molecular Design of a Series of 4-Aminoquinazolines as Inhibitors of Aurora B Kinase. Chinese Journal of Chemistry. 2011, 29: 1785-1799

44. Deng D W, Zhang W H, Chen X Y, Liu F, Zhang J, Gu Y Q, et al. Facile Synthesis of High-Quality, Water-Soluble, Near-Infrared-Emitting PbS Quantum Dots. European Journal of Inorganic Chemistry. 2009: 3440-3446.

45. Yang Z, Chang L, Chiang C L, and James Lee L. Micro-/nano-elec-troporation for active gene delivery. Current pharmaceutical design. 2015, 21: 6081-6088

46. Chen Y, Zhang W, Huang Y, Gao F, Sha X, and Fang X. Pluronic-based functional polymeric mixed micelles for co-delivery of doxorubicin and paclitaxel to multidrug resistant tumor. International Journal of Pharmaceutics. 2015, 488: 44-58.

47. Shi C, Zhang Z, Shi J, Wang F, and Luan Y. Co-delivery of docetaxel and chloroquine via PEO–PPO–PCL/TPGS micelles for overcoming multidrug resistance. International Journal of Pharmaceutics. 2015, 495: 932-939.

48. Ma Y, Liu D, Wang D, Wang Y, Fu Q, Fallon J K, et al. Combina-tional Delivery of Hydrophobic and Hydrophilic Anticancer Drugs in Single Nanoemulsions To Treat MDR in Cancer. Molecular Pharma-ceutics. 2014, 11: 2623-2630.

49. Zhong X, Bailey N A, Schesing K B, Bian S D, Campos L M, and Braunschweig A B. Materials for the preparation of polymer pen litho-graphy tip arrays and a comparison of their printing properties. Journal of Polymer Science Part a-Polymer Chemistry. 2013, 51: 1533-1539.

50. Zhu H, Chen H, Zeng X, Wang Z, Zhang X, Wu Y, et al. Co-delive-ry of chemotherapeutic drugs with vitamin E TPGS by porous PLGA nanoparticles for enhanced chemotherapy against multi-drug resis-tance. Biomaterials. 2014, 35: 2391-2400.

51. Patel N R, Rathi A, Mongayt D, and Torchilin V P. Reversal of mul-tidrug resistance by co-delivery of tariquidar (XR9576) and paclitaxel using long-circulating liposomes. International Journal of Pharmaceu-tics. 2011, 416: 296-299.

52. Kim S H, Sun Y, Kaplan J A, Grinstaff M W, and Parquette J R. Photo-crosslinking of a self-assembled coumarin-dipeptide hydrogel. New Journal of Chemistry. 2015, 39: 3225-3228

53. Collins K A, Zhong X, Song P, Little N R, Ward M D, and Lee S S. Electric-Field-Induced Reversible Phase Transitions in Two-Dimensional Colloidal Crystals. Langmuir. 2015, 31: 10411-10417.

54. Qin M, Lee Y E, Ray A, and Kopelman R. Overcoming cancer multidrug resistance by codelivery of doxorubicin and verapamil with hydrogel nanoparticles. Macromolecular bioscience. 2014, 14: 1106-1115.

55. Fan L, Li F, Zhang H, Wang Y, Cheng C, Li X, et al. Co-delivery of PDTC and doxorubicin by multifunctional micellar nanoparticles to achieve active targeted drug delivery and overcome multidrug resistance. Biomaterials. 2010, 31: 5634-5642.

56. Wang J, Ma W, and Tu P. Synergistically Improved Anti-tumor Ef-ficacy by Co-delivery Doxorubicin and Curcumin Polymeric Micelles.

Macromolecular bioscience. 2015, 15: 1252-1261.

57. Misra R, and Sahoo S K. Coformulation of doxorubicin and curcumin in poly(D,L-lactide-co-glycolide) nanoparticles suppresses the development of multidrug resistance in K562 cells. Molecular pharma-ceutics. 2011, 8: 852-866.

58. Wang Y, Ding Y, Liu Z, Liu X, Chen L, and Yan W. Bioactive lipids-based pH sensitive micelles for co-delivery of doxorubicin and ce-ramide to overcome multidrug resistance in leukemia. Pharmaceutical research. 2013, 30: 2902-2916.

59. Sun W, Fang M, Yang Z, Chen Y, Xiao Y, Wan M, et al. Delivery System of CpG Oligodeoxynucleotides through Eliciting an Effective T cell Immune Response against Melanoma in Mice. Journal of Cancer. 2016, 7: 241-250

60. Yu B, Wang X, Zhou C, Teng L, Ren W, Yang Z, et al. Insight into mechanisms of cellular uptake of lipid nanoparticles and intracellular release of small RNAs. Pharmaceutical Research. 2014, 31: 2685-2695.

61. Hu J, and Gao D Z. Distinction immune genes of hepatitis-induced heptatocellular carcinoma. Bioinformatics. 2012, 28: 3191-3194

62. Hu J, and Gao Z. Modules identification in gene positive networks of hepatocellular carcinoma using Pearson agglomerative method and Pearson cohesion coupling modularity. Journal of Applied Mathema-tics. 2012, 2012

63. Taratula O, Kuzmov A, Shah M, Garbuzenko O B, and Minko T. Nanostructured lipid carriers as multifunctional nanomedicine platform for pulmonary co-delivery of anticancer drugs and siRNA. Journal of Controlled Release. 2013, 171: 349-357.

64. Biswas S, Deshpande P P, Navarro G, Dodwadkar N S, and Torchilin V P. Lipid modified triblock PAMAM-based nanocarriers for siRNA drug co-delivery. Biomaterials. 2013, 34: 1289-1301.