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REVIEW

Nanocarrier-mediated co-delivery of chemotherapeutic drugs and gene agents for cancer treatment

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Abstract The efficacy of chemotherapeutic drug in cancer treatment is often hampered by drug resistance of tumor cells, which is usually caused by abnormal gene expression. RNA interference mediated by siRNA and miRNA can selectively knock down the carcinogenic genes by targeting specific mRNAs. Therefore, combining chemotherapeutic drugs with gene agents could be a promising strategy for cancer therapy. Due to poor stability and solubility associated with gene agents and drugs, suitable protective carriers are needed and have been widely researched for the co-delivery. In this review, we summarize the most commonly used nanocarriers for co-delivery of chemotherapeutic drugs and gene agents, as well as the advances in co-delivery systems.

Abbreviations: γ-CD, γ-cyclodextrin; ANG-CLP, angiopep-2 modified cationic liposome; CMC, critical micelle concentration; CPLA, cationic polylactide; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; FA, folic acid; FCAP, ferrocenium capped amphiphilic pillar[5]arene; GSH, glutathione; miRNA, micro-RNA; OEI, oligoethylenimine; PAMAM, poly(amido amine); PAsp(AED), poly(*N*-(2,2′-dithiobis(ethylamine))aspartamide); PCL, poly(ε-caprolactone); PDMAEMA, polydimethylaminoethyl methacrylate; PDPA, poly(2-(diisopropyl amino)ethyl methacrylate); PEG, polyethyleneglycol; PEI, poly(ethyleneimine); PEI-Fc, ferrocene modified poly(ethyleneimine); PEI-PCHLG, poly(ethylene imine)-poly(γ-cholesterol-L-glutamate); PEI-PCL, poly (ethyleneimine) and poly(ε-caprolactone); PLA, polylactic acid (or polylactide); PLGA, poly(lactic-*co*-glycolic acid); P*n*BA, poly(*n*-butyl acrylate); PPEEA, poly(2-aminoethyl ethylene phosphate); RNAi, RNA interference; siRNA, small interfering RNA; siVEGF, VEGF-targeted siRNA; SNPs, supramolecular nanoparticles; SSTRs, somatostatin receptors poly(*N*-(2,2′-dithiobis(ethylamine))aspartamide)

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1. Introduction

Cancer is one of the most devastating diseases and a leading cause of death in the world. According to the mortality data from the National Center for Health Statistics in 2013, one in four deaths in the United States is due to cancer[1]. Chemotherapy is a treatment choice for many types of cancers, but its success is often hampered by development of drug resistance after repeated administration. Drug resistance has a genetic basis and it is caused by abnormal gene expression. There are several types of drug resistance, including efflux pumps which reduce the cellular concentration of the drug, alterations in membrane lipids that reduce cellular uptake, increased or altered drug targets, metabolic alteration of the drug, inhibition of apoptosis, repair of the damaged DNA, and the alteration of cell cycle checkpoints[2–5].

RNA interference (RNAi) is a special mechanism which occurs normally in most eukaryotic cells. RNAi mediated by small interfering RNA (siRNA) and microRNA (miRNA) have emerged as the most promising strategies for anti-cancer therapy, since siRNA and miRNA can induce gene-specific cleavage through their complementary pairing with mRNA, resulting in degradation of mRNA. For example, siRNA targeting the *MDR1* gene can reduce the formation of efflux transporters in cell membrane, resulting in an increase in cellular drug concentration[6]. Survivin siRNA can sensitize the drug resistance cells by inhibiting cell survival pathway[7]. Therefore, the silencing of the gene will open a window of time in which the resistant cells transiently become sensitized to the anti-cancer drug, thereby overcoming multi-drug resistance[8–11]. On the other hand, since tumor suppressor protein gene, such as *p53*, can induce cell growth arrest or apoptosis, plasmid DNA encoding *p53* can also be delivered for cancer therapy. All the RNA interference agents and plasmid DNA are known as gene agents[12].

Combination therapy is emerging as a promising approach for the treatment of cancer. Rational drug combinations aim to exploit either additive or synergistic effects arising from the action of several species with the final goal to maximize therapeutic efficacy. It has been shown that an appropriate combination of chemotherapeutic drugs and gene agents can improve the therapeutic outcome and patient compliance due to reduced dose and decreased development of drug resistance[13,14].

However, the biggest challenge in co-delivery drugs and gene agents is to find applicable carriers, since gene agents have higher molecular weight and negatively charged surface, while most frequently used anti-cancer drugs are hydrophobic small molecules[15]. During the recent years, there has been a remarkable progress in a co-delivery system. The objective of this article is to review various nanocarriers that have been researched for the co-delivery of chemotherapeutic drugs and gene agents for tumor therapy, and to make suggestions for further design the in co-delivery system.

2. Co-delivery nanocarriers for drugs and gene agents

Since the physicochemical properties of oligonucleotides are drastically different from those of small molecular weight drugs, separate mechanisms are usually required to encapsulate these two distinct payloads. The small-molecule drugs can be enclosed within the nanocarriers *via* hydrophobic force, electrostatic interaction or chemical conjugation, whereas gene agents are usually compressed by the carriers through electrostatic force[15]. To meet the above requirements, traditional nanocarriers, such as liposome and micelle, and novel nanocarriers, including dendrimer and a supramolecular system, have been used to delivery chemotherapeutic drugs and gene agents, as demonstrated in Table 1[6,7,16–39] and 2[40–60]. The following section will systematically review the commonly and recently employed organic nanocarriers for the co-delivery of gene agents and drugs.

2.1. Traditional nanocarrier

2.1.1. Liposome-based nanocarrier

Liposomes represent one of the most successful drug vehicles, as well as in the co-delivery of chemotherapeutic drugs and gene agents. Recently, many researchers reported their achievements in co-delivery systems using modified cationic liposomes, as shown in Table 1. 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP) is the most commonly utilized cationic lipid[21]. Cationic liposome-based nanoformulations are usually prepared through simple electrostatic interaction between the positively charged cationic lipids and the negatively charged phosphate backbones of oligonucleotides. The drugs can be loaded *via* hydrophobic force, as exhibited in Fig. 1a. However, the liposome usually has poor physiological stability compared to other polymeric vectors[13].

Saad and colleagues[19] demonstrated a cationic liposome-based co-delivery system, which consisted of cationic lipids, doxorubicin, and siRNA targeted to *MRP1* and *BCL2* mRNA (suppressors of pump and nonpump cellular resistance, respectively). The drug vehicle provided an effective co-delivery approach to induce cell death and to suppress cellular resistance in MDR lung cancer cells. Sun and colleagues[17] reported an angiopep-2 modified cationic liposome (ANG-CLP) for the efficient co-delivery of a therapeutic gene encoding the human tumor necrosis factor-related apoptosis-inducing ligand (*pEGFP-hTRAIL*) and paclitaxel for glioma. The dual targeting co-delivery system improved uptake and gene expression not only in U87 MG cells and BCECs, but also in the glioma bed and infiltrating margin of intracranial U87 MG glioma-bearing models.

To improve tumor therapy efficacy, Feng and colleagues[20] built a vapreotide-modified core-shell type nanoparticle co-encapsulating VEGF-targeted siRNA (siVEGF) and paclitaxel. Vapreotide is a somatostatin analog possessing high affinity to somatostatin receptors (SSTRs), which are overexpressed in many tumor cells. The nanoparticle core was a negatively charged ternary complex composed of siRNA, chondroitin sulfate and protamine, and could be coated with cationic lipid shell. As a result, the mixed liposome had significantly stronger drug distribution in tumor tissues *via* receptor-mediated targeting delivery, accompanied by substantial inhibition of neovascularization induced by siVEGF silencing.

2.1.2. Micelle based nanocarrier

To achieve simultaneous delivery of chemotherapeutic drugs and gene agents, the carriers must be able to protect the contents from degradation and prevent premature release. Micelleplexes consisting of amphiphilic block copolymers are the most commonly reported examples of co-delivery carriers, as shown in Table 1. Usually, the micelle self-assembles with the hydrophobic blocks to form the interior of the micelle and with hydrophilic blocks to form the micelle shell[61]. The hydrophobic interior acts as a reservoir for the poorly soluble hydrophobic drugs. Hydrophilic blocks on the shell mask the payloads. The most frequently used mask is polyethyleneglycol (PEG). Other commonly-used hydrophilic blocks are cationic polymers that can condense/complex

Table 1 Traditional co-delivery nanocarriers of chemotherapeutic drugs and gene agents in recent researches.

Carrier type	Composition of carrier	Drug	Gene agent	Cell line	Ref.
Cationic liposome	Cationic solid lipid nanoparticles (cSLN)	Paclitaxel	MCL1 siRNA	KB	16
	Angiopep-2 modified cationic liposome	Paclitaxel	*pEGFP-hTRAIL*	U87	17
	PLGA/FPL	Doxorubicin	*pEGFP*	MDA-MB-231	18
	Cationic liposome	Doxorubicin	MRP1 and BCL2 siRNA	MCF-7, HCT15	19
	Vapreotide-modified core–shell liposome	Paclitaxel	VEGF siRNA	MCF-7	20
	Lipid nanocapsules functionalized with PEI	Paclitaxel	pDNA	HEK	21
	Thermosensitivemagneticcationic liposomes	Doxorubicin	SATB1 shRNA	MKN-28	22
	Nanostructured lipid carrier	Doxorubicin or paclitaxel	MRP1 and BCL2 siRNA	A549	23
	PEGylated liposome	Docetaxel	BCL2 siRNA	A549	24
Micelle	Amphiphilic chimeric peptide $(Fmoc)_2KH_7$-TAT	Doxorubicin	*p53* plasmid	293T, Hela	25
	PEG-PAsp(AED)-PDPA	Doxorubicin	BCL2 siRNA	SKOV-3	26
	PEI-PCHLG	Docetaxel	pDNA	HEK293	27
	PDMAEMA-PCL-PDMAEMA	Paclitaxel	GFP siRNA	MDA-MB-435	28
	P85-PEI/TPGS	Paclitaxel	Survivin shRNA	A549	29
	ABP-$PEG_{3.5k}$-paclitaxel	Paclitaxel	*gWiz-Luci*	MCF-7, A549	30
	FA-PEG-PGA and PEI-PCL	Doxorubicin	BCL2 siRNA	C6	31
	FA-PEG-PGA and PEI-PCL	Doxorubicin	BCL2 siRNA	Bel-7402	32
	PEO-*b*-PCL	Doxorubicin	MDR1 siRNA	MDA-MB-435	6
	Oligopeptide amphiphile	Doxorubicin	Luc siRNA	HepG2	33
	PDP-PDHA	Doxorubicin	Surviving shRNA	MCF-7	34
	PEG-pp-PEI-PE	Paclitaxel	Surviving siRNA	A549	7
	MPEG-PCL-g-PEI	Doxorubicin	Msurvivin T34A gene	B16F10, MCF-7, CT26	35
	PEOz-PLA-g-PEI	Doxorubicin	mcDNA	MCF-7	36
	PEG-PLL-PLLeu	Docetaxel	BCL2 siRNA	MCF-7	37
	Cationic core-shell nanoparticles	Paclitaxel	*IL-2* plasmid BCL2 siRNA	MDA-MB-231, 4T1	38
	mPEG45-*b*-PCL80-*b*-PPEEA10	Paclitaxel	polo-likekinase 1 (Plk1) specific siRNA	MDA-MB-435	39

with negatively charged DNA or RNA. Poly(ethyleneimine) (PEI) and poly(2-aminoethyl ethylene phosphate) (PPEEA) are the most frequently used cationic blocks. The most popular hydrophobic polymers are poly(ε-caprolactone) (PCL), poly(*n*-butyl acrylate) (P*n*BA), polylactide (PLA) and poly(lactic-*co*-glycolic acid) (PLGA). The outer shell may be further decorated with targeting ligands, such as folate, to enhance the active targeting-ability of the carrier in most cases[13], as exhibited in Fig. 1b. Micelle-based nanocarriers are tunable, biocompatible, and physiologically stable owing to their low critical micelle concentration (CMC); the preparation process of functional block polymers is always complicated[13].

Zhu and co-workers[28] prepared a biodegradable cationic micelle with PDMAEMA-PCL-PDMAEMA triblock copolymer, which formed nano-sized micelles in water with positively charged surface that could be applied for the delivery of VEGF siRNA and paclitaxel. Cao and colleagues[32] synthesized a diblock copolymer consisted of linear poly(ethyleneimine) and poly(ε-caprolactone) (PEI-PCL), and the amphiphilic polymer assembled into micelles for co-delivery of BCL2 siRNA and doxorubicin. Folic acid was conjugated to the polyanion and further coated onto the surface of the cationic PEI-PCL nanoparticle pre-loaded with siRNA and doxorubicin, potentiating a ligand-directed delivery to human hepatic cancer cells. This hierarchical assembly strategy was beneficial for active targeting. Another dual-functional poly(ethyleneimine)-poly(γ-cholesterol-L-glutamate)(PEI-PCHLG) copolymer was synthesized by Zhang et al.[27] for the co-delivery. PCHLG played an analogous role to lipoproteins in terms of drug delivery, and had high drug loading. PEI-PCHLG was able to assemble into micelles with high drug and gene loading efficiency. Amphiphilic chimeric peptide, for example, $(Fmoc)_2KH_7$-TAT[25] and Ac-$(AF)_6$-H_5-K_{15}-NH_2(FA32)[33], can also be used for drug and gene co-delivery.

Stimuli-responsive micelle systems have also been developed for co-delivery. Zhu et al.[7] presented a simple but multifunctional micellar platform constructed by a matrix metalloproteinase 2 (MMP2)–sensitive copolymer (PEG-pp-PEI-PE) *via* self-assembly for tumor-targeted siRNA and drug co-delivery. The unique delivery system exhibited excellent stability and tumor-targeting triggered by the up-regulated tumoral MMP2. This system achieves enhanced cell internalization after MMP2-activated exposure of the previously hidden PEI. Chen et al.[26] developed a reduction and pH dual-sensitive nanocarrier for synergistic cancer therapy. A ternary block copolymer PEG-PAsp(AED)-PDPA contained pH-sensitive poly(2-(diisopropyl amino)ethyl methacrylate) (PDPA), reduction-sensitive poly(*N*-(2,2′-dithiobis(ethylamine))aspartamide) (PAsp(AED)) and PEG. The copolymer assembled into a core–shell structural micelle, which encapsulated doxorubicin in its pH-sensitive core and BCL2 siRNA in a reduction sensitive interlayer. The dual stimuli-responsive design of micellar carrier allowed microenviroment-specific rapid release of both doxorubicin and BCL2 siRNA inside acidic

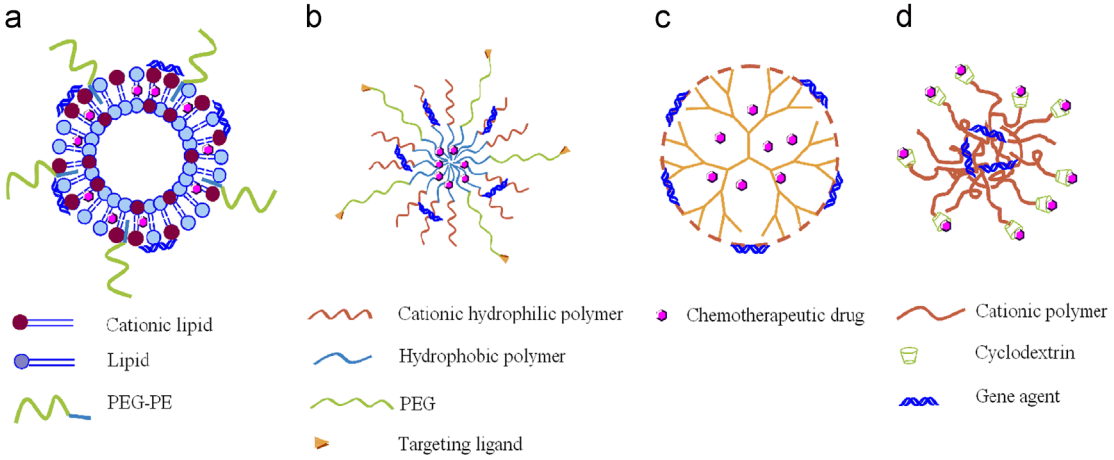

Figure 1 Schematic illustration of four major types of nanocarriers to co-delivery gene and chemotherapeutic drug. (a) Cationic liposome, the most frequently used cationic lipid and general lipid are DOTAP and DOPE, respectively, and PEG modified with PE can prolong the cycle time in the circulation system. (b) Micelle system, hydrophilic block is usually positively charged, such as PEI, polyamino acid and so on, PCL, PLA and PE are employed as hydrophobic core. (c) Dendrimer system, PAMAM is the most commonly used dendrimer for co-delivery. (d) A supramolecular system, γ-CD can form inclusion complexes with chemotherapeutic drugs.

lysosomes with enriched reducing agent. This resulted in synergistically-enhanced apoptosis of human ovarian cancer SKOV-3 cells, thereby dramatically inhibiting tumor growth.

2.2. *Non-traditional nanocarrier*

2.2.1. *Dendrimer based nanocarrier*

Dendrimers are hyperbranched and monodispersed macromolecules which have defined molecular weights and host–guest entrapment properties. More importantly, dendrimers can interact with drug and gene molecules by simple encapsulations, electrostatic interactions and covalent conjugations since they possess empty internal cavities and a much higher density of surface functional group[62], as shown in Fig. 1c. Therefore, monodispersal and high drug-loading capacity are prominent advantages of dendrimers. However, dendrimers still have some safety–toxicity issues according to comprehensive statistics[14].

Several polyamine polymers have been explored as carriers for drug delivery. For example, poly(amido amine) (PAMAM), a cationic dendrimer which introduces ammonia as the core, has been investigated as non-viral delivery vector for efficient siRNA delivery[63]. Han and co-workers[41] employed peptide HAIYPRH (T7)-conjugated PEG-modified PAMAM dendrimer (PAMAM-PEG-T7) for the co-delivery of pDNA and doxorubicin. In comparison with single doxorubicin or pDNA delivery system, this co-delivery system induced apoptosis of tumor cells *in vitro* and inhibited tumor growth *in vivo* more efficiently. Combining PAMAM with other amphiphilic block copolymers was also an approach for co-delivery. Biswas et al.[46] modified PAMAM with poly(ethyleneglycol)-1,2-dioleoyl-sn-glycero-3-phospho-ethanolamine to form a new construct G(4)-D-PEG-2K-DOPE. This G(4)-PAMAM dendrimer was utilized as a cationic source for efficient siRNA condensation; DOPE provided optimum hydrophobicity and compatible cellular interaction for enhanced cell penetration. PEG rendered flexibility to the G(4)-D for easy accessibility of siRNA for condensation. This nanocarrier formed stable polyplexes with siRNA, showed a significantly higher cellular uptake of siRNA, excellent serum stability and efficient micellization, and higher doxorubicin-loading efficiency.

Except for PAMAM, dendrimer analogs have also been investigated in drug and gene co-delivery. Liu et al.[42] prepared a new cyclodextrin derivative (CD-PLLD) consisted of a β-cyclodextrin core and poly(L-lysine) dendron arms for doxorubicin and MMP-9 siRNA plasmid co-delivery. Qian et al.[43] constructed dendrimer analogs with three amphiphilic star-branched copolymers comprising polylactic acid (PLA) and polydimethylaminoethyl methacrylate (PDMAEMA) for microRNA and doxorubicin transport. By testing architectures with different repeat degrees, they found that $(AB_3)_3$ architecture exhibited the highest transfection efficiency. Ma and colleagues[44] designed a star-shaped porphyrin-arginine-functionalized poly(L-lysine) copolymer (PP-PLLD-Arg) for photo-enhanced drug and gene co-delivery. Results with this copolymer demonstrated that PP-PLLD-Arg with suited irradiation was a promising non-toxic and photo-inducible effective drug and gene delivery strategy.

2.2.2. *Supramolecular nanocarrier*

The development of self-assembly techniques has permitted the introduction of supramolecular nanoparticles (SNPs), such as host-guest architectures, as drug and non-viral gene carriers. The host-guest system is a complex in which one chemical compound (the "host") forms a cavity in which molecules of a second "guest" compound are located. In drug delivery system, the most frequently used host is γ-cyclodextrin (γ-CD), which contains a torus-like structure with a hydrophobic cavity, and can form inclusion complexes with chemotherapeutic drug, as demonstrated in Fig. 1d. Rational inclusion complexes exhibit excellent serum stability and promising application. However, perfectly matched host/guest materials are not easy to find.

Recently, Yang and colleagues[49] designed a pH-responsive drug/gene co-delivery nanoplatform by means of host–guest chemistry. γ-CD/doxorubicin complexes were attached onto phenylboronic-acid-modified oligoethylenimine ($PEI_{1.8K}$-$PB_{2.9}$) at neutral conditions. The drug is detached from $PEI_{1.8K}$-$PB_{2.9}$ under acidic conditions owing to the acidity-labile feature of

boronate linkage, thereby facilitating drug release. Moreover, $PEI_{1.8K}$-$PB_{2.9}$-γ-CD conjugates demonstrated significantly improved cell-biocompatibility and DNA transfection activity by overcoming serum-susceptible drawbacks frequently associated with synthetic gene carriers. Zhao and co-workers[12] also employed γ-CD and multiple oligoethylenimine (OEI) arms with folic acid (FA) as co-delivery materials for paclitaxel and pDNA.

In another study, Fan and colleagues[48] designed a SNP consisted of host PEI-CD (as gene vector) and guest adamantane conjugated groups (as chemotherapeutic agent carriers) for co-delivery of drug and gene. The adamantane-conjugated doxorubicin as the guest Ad-Dox component assembled with the host PEI-CD into supramolecular PEI-CD/Ad-Dox, which could further interact with plasmid DNA to form drug- and gene-loaded PEI-CD/Ad-Dox/pDNA SNP. The *in vitro* data in different cell lines indicated that such SNP could ensure that both drug and gene can be delivered to the same cancer cell, providing the feasibility of combinational tumor treatment. Hu et al.[47] conducted synergistic treatment of ovarian cancer by co-delivery of survivin shRNA and paclitaxel *via* a similar supramolecular micellar assembly.

2.2.3. *Novel nanoformulation*

Chang et al.[50] constructed a redox-responsive system for drug/siRNA co-delivery based on ferrocenium capped amphiphilic pillar[5]arene (FCAP). Pillar[*n*]arenes are a new class of macrocyclic compounds which possess a hydrophobic core sandwiched between two functional rims and can self-assemble to cationic vesicles in aqueous solution. The ferrocenium cation, which is sensitive to glutathione (GSH), is a redox-responsive bond, and the positive charge of ferrocenium makes possible for the loading of negatively charged siRNA onto nanocarriers. Therefore, FCAP allowed building an ideal GSH-responsive drug/siRNA co-delivery system for rapid drug release and gene transfection in cancer cells in which higher GSH concentration existed.

Chen et al.[59] reported a unique architecture, cationic polymeric nanocapsule, which had well-defined covalently stabilized biodegradable structures and can function as a potentially universal and safe therapeutic nanocarrier for co-delivery of doxorubicin and siRNA targeting interleukin-8. This nanocapsule was synthesized from allyl-functionalized cationic polylactide (CPLA) by a highly efficient UV-induced thiol-ene interfacial cross-linking in transparent miniemulsions. Liu and co-workers[56] adopted a double-emulsion solvent evaporation technique to prepare intelligent gelatinases-stimuli nanoparticles for the co-delivery of miR-200c and docetaxel. This miniemulsion was able to inhibit cancer stem cells and non-cancer stem cells and showed promise for cancer therapy.

Dr. Hammond's group[64] developed a layer by layer nanoplatform for systemic co-delivery of doxorubicin and siRNA for potential triple-negative breast cancer treatment. The layer by layer nanoparticle could be divided into three parts in structure: drug-loaded core, siRNA/polycation–loaded middle film and tumor targeting outer shell. The advantage of this unique architecture was that it provided a modular platform for a broad range of controlled multidrug therapies customizable to the cancer type in a singular nanoparticle delivery system. Meanwhile, Sun et al.[58] presented a system with multilayers for co-delivery of doxorubicin and DNA. Ferrocene modified poly(ethyleneimine) (PEI-Fc) formed micelles in solution and trapped DNA and drug to form PEI-Fc–DOX-DNA nanocomplexes, and such cationic nanocomplexes were further used to construct multilayers through layer by layer assembly with

Table 2 Non-traditional co-delivery nanocarriers of chemotherapeutic drugs and gene agents in recent researches.

Carrier type	Composition of carrier	Drug	Gene agent	Cell line	Ref.
Dendrimer	T7-modified dendrigraftpoly-L-lysine	Doxorubicin	*pTRAIL*	U87	40
	PAMAM-PEG-T7	Doxorubicin	*pORF-hTRAIL*	Bel-7402	41
	b-cyclodextrin core and poly(L-lysine) dendron arms	Docetaxel	*pMR3*	HNE-1	42
	PLA-*b*-PDMAEMA	Doxorubicin	miR-21	LN229	43
	Porphyrin-arginine Functionalized poly(L-lysine) copolymer	Docetaxel	MMP-9 shRNA	HNE-1	44
	Poly(L-lysine) dendrimers with a silsesquioxane cubic core	Doxorubicin	luciferase siRNA	U87	45
	G(4)-D-PEG-2K-DOPE	Doxorubicin	siGFP	A549	46
Supramolecular system	Host PEI-CyD (PC) guest adamantine conjugated PTX	Paclitaxel	Survivin shRNA	SKOV3	46
	Host PEI-CyD (PC) guest adamantine conjugated DOX	Doxorubicin	*pTRAIL*	SKOV3	48
	β-CD and OEI-FA	Paclitaxel	*p53*	KB, A549	12
	$PEI_{1.8k}$–$PB_{2.9}$–γ-CD	Doxorubicin	pDNA	293T, HeLa	49
Novel nanoformution	Amphiphilicpillar[5]arene capped with ferrocenium	Doxorubicin	MDR1 siRNA	293T, HeLa	50
	Aptamerconjugated PEI-PEG	Doxorubicin	Bcl-xL shRNA	PC3, LNCaP	51
	Chitosan-graft-PEI	Candesartan	*p53*	PANC-1	52
	Hyaluronic acid and chitosan	Doxorubicin	miR-34a	MDA-MB-231	53
	Layered double hydroxide	5-fluorouracil	Allstars Cell Death siRNA	MCF-7, U2OS and HCT-116	54
	CholsiRNA/LDL-coupled *N*-succinyl chitosan	Doxorubicin	MDR1 siRNA	HepG2	55
	PEG-Pep-PCL copolymer	Docetaxel	miR-200c	BGC-823	56
	PLGA nanoformulation	Doxorubicin	MDR1 siRNA	MCF-7	57
	PEI-Fc	Doxorubicin	DNA	HepG2	58
	Cationic polymeric nanocapsules	Doxorubicin	IL-8 siRNA	MCF-7	59
	PEI-PEG based nanoparticles	Doxorubicin	DNA	HUVE, HepG2, MCF-7	60

negatively charged dextran sulfate. The multilayers could be potentially applied to the biomedical devices for cancer treatment, regenerative medicine, *etc*. Some other novel co-delivery nanoformulations are displayed in Table 2.

3. Conclusions and future perspectives

The co-delivery of chemotherapeutic drugs and gene agents provides a promising strategy to overcome drug resistance in cancer therapy. According to recent research, it is clear that combination delivery of gene and drug using nanocarriers is indeed helpful in inhibiting tumor growth compared to gene or drug alone. Although various nanocarriers have been developed for co-delivery, most carriers just focus on successful co-delivery of gene and drug. This approach has often resulted in functional materials, such as PEG, PEI and PLGA et al., being used repeatedly in different permutation and combination, without paying attention to the rational ratio of gene and drug or the interaction between them in the vehicle. Development of new materials and technologies affords the opportunity to discover and produce novel drug delivery systems. Presently, an ideal co-delivery carrier should be biocompatible and biodegradable, and demonstrate circulatory stability, thereby facilitating transport of the cargos to the targeting sites. The ideal carrier will also be multifunctional, with the ability to transport simultaneously both chemotherapeutic drugs and gene agents to cancer cells, releasing the payloads in a controlled manner and accurate dose, thereby achieving a maximum effect of the combination therapy for treating drug resistant tumors. Further studies should focus on the interaction between drugs and gene agents, as well as the interaction between therapeutic agents and carriers. Continuous development of such combination delivery systems will ultimately lead toward availability of effective therapies for cancer.

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