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Functional Nanoplatforms for Enhancement of Chemotherapeutic Index

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Abstract: Recently, the advancement of nanotechnology has had a significant impact on clinical therapeutics. Advances in biocompatible drug carriers using organic/inorganic nanoparticles have enabled safer and more efficient delivery of anti-cancer agents. In particular, nanoparticles designed and fabricated by sophisticated processes can (as drug carriers) deliver precise doses of multiple anti-cancer agents to target cancer cells, improving therapeutic efficacy and minimizing side effects. In addition, a comprehensive understanding of cancer biology, chemo-resistance, cancer relapse, and metastasis is essential for successful development of combination therapy using nanoparticles. In this review, we discuss the species of functional nanoparticles for delivery of anti-cancer agents and their potential to improve chemotherapeutic indices *via* combination therapy. We also discuss novel therapeutic strategies using functional nanoparticles developed through multidisciplinary collaboration for combination chemotherapy.

Keywords: Anti-cancer agents, Cancer, Combination chemotherapy, Nanoparticle, Synergistic effect.

1. PROBLEMS IN CHEMOTHERAPEUTIC DRUG ADMINISTRATION

Cancer develops *via* a multistep carcinogenesis process with highly incomprehensible and complex signaling pathways under numerous cellular physiological systems [1, 2]. Moreover, certain cancer cells from primary tumors are prone to spread to distant sites within the body, complicating treatment of the disease. Thus, chemotherapeutic agents used for the ablation of cancerous cells have been developed as synthesized chemicals for several decades [3]. For effective cancer therapy, the key issue is to establish the optimal dose of chemotherapeutic agents in tumor sites for the destruction of cancerous cells with minimal side effects to normal cells surrounding the tumor sites. In particular, the proper chemotherapeutic agent should be selected on the basis of the origin of the cancer and the desired cytotoxic mechanism. Despite advances in the diagnosis and treatment of cancer for decades, however, cancer is still a leading cause of mortality worldwide. Recently, organic/inorganic nanoplatforms have emerged as nanomedicines for overcoming biological barriers and improving cancer therapy by efficiently delivering chemotherapeutic agents and reducing adverse effects [4-6]. General chemotherapeutic agents that are loadable onto nanoplatforms are listed in Table **1**. The molecular weight and solubility of the selected chemo-therapeutic agent and the compatibility between the chemo-therapeutic agent and the nanomaterial are key elements in loading onto nanoplatforms. In particular, lipid nanoplatforms such as liposomes are already widely used in clinics while polymeric nanoplatforms such as micelles are in clinical trials in several countries [7-10]. Moreover, functionalized nanoplatforms, conjugated to moieties that enhance site-specific delivery and tailored release, have been developed [11, 12]. Due to tumor heterogeneity and the complexity of the tumor microenvironments, which impede successful treatment of cancer, novel and advanced approaches enabling reversion of multidrug resistance (MDR) and treatments that act through multiple pathways with fewer adverse effects are desperately needed [13]. In this review, we describe various strategies for the enhancement of chemotherapeutic indices using functional nanoplatforms (Fig. **1**). The importance as well as the current limitation of chemotherapeutic agents for cancer treatment is first introduced. Subsequently, we highlight functional nanoplatforms using organic or inorganic nanomaterials for the systemic incorporation and effective delivery of chemotherapeutic drugs to the tumor site. In particular, we address recent advances in chemosensitization methods based on functional nanoplatforms aimed at overcoming numerous biological barriers of cancer. We also discuss the potential for functional nanoplatforms to overcome clinical and technical hurdles for well-tailored personalized cancer therapy.

2. NANOPLATFORMS AND CLASSIFICATION

For a few decades, scientific research has aimed to accelerate the development of novel nanotechnologies. In particular, the remarkable effect that nanoscale materials possess properties distinct from bulk materials may be exploited in the biomedical fields. Moreover, the use of nanotechnology in medicine has changed the foundations of cancer diagnosis and therapy. The extremely small dimensions (diameters of 10–200 nm, typically) of nanomaterials have proven useful in a range of different approaches to cancer diagnosis and therapy, such as recognition of molecular biomarkers, disease imaging, targeted drug delivery, and convergence systems for simultaneous therapeutic and diagnostic applications. To achieve these objectives, a number of key properties of nanoparticles (colloidal size, surface charge, payload density, circulation time in the blood, and target ligands) should be tailored to the biological context and the available medical information. To enhance the effectiveness of cancer chemotherapy, the next key elements are integrated into the nanoplatforms: 1) the working mechanism for the selected chemotherapeutic agent, 2) the clinical information for the target cancer, 3) the foundations of cancer biology, and 4) the physico-chemical properties of hydrophilic and/or hydrophobic chemotherapeutic agents. In the next section, we introduce several forms of nanoplatforms, including lipid nanostructures, synthetic polymer nanostructures, and inorganic nanostructures such as silica, magnetic, and carbon nanostructures that achieve efficient drug loading and delivery to the tumor site (Table **2**).

2.1. Organic Nanoplatforms

2.1.1. Liposomes

Liposomes based on phospholipids have been developed for cancer treatment due to biocompatibility and the ability to enhance the solubility of chemotherapeutic agents [25, 26]. In particular,

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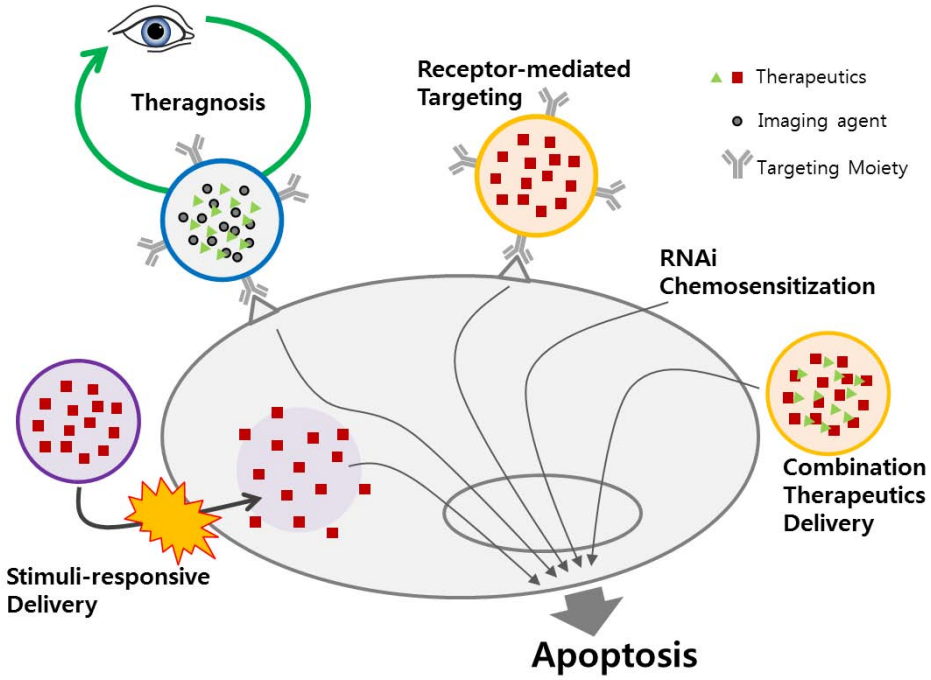

Fig. (1). Schematic illustration of various strategies for the enhancement of chemotherapeutic indices using functional nanoplatforms.

Table 1. Various Chemotherapy Agents Loadable by Nanoplatforms and their Mechanism [3]

Category	Common Name	Target	Mechanism	Ref.
Alkylating Agents	cyclophosphamide	DNA	nitrogen mustard (reactive)	[14]
	cisplatin (CDDP)	DNA	platinum coordination (reactive)	[15, 16]
Antibiotics	doxorubicin (DOX, adriamycin)	DNA	polycyclic rings allow intercalation (interactive), quinones allow redox reactions (reactive)	[17-19]
Antimetabolites	methotrexate	dihydrofolate reductase	mimics folate interactive)	[20]
	5-fluorouracil (5-FU)	thymdylate synthase, also incorporated into RNA and DNA	mimics deoxyuridine triphosphate (reactive and interactive)	[21]
	paclitaxel (Taxol)	microtubules	inhibits tubule depolymerization (reactive)	[22]
	etoposide	topoisomerase II	inhibits reconnection of DNA (interactive)	[23]
Hormones	dexamethasone	glucocorticoid receptor	modify DNA transcription (interactive)	[24]

polyethylene glycol-modified (PEGylated) liposomal DOX (Doxil) is a successful drug delivery system in the cancer therapy market [56]. Liposomal Doxil exhibited the most prolonged circulation to date, with a terminal half-life of 55 hours in humans, due to the "stealth" property of liposomes. For several decades, functional liposomes, generated by the modification of composition and surface chemistry, have been established as effective cancer therapeutic agents. For targeted delivery of liposome loaded with chemotherapeutic agents to tumor tissue, tumor-specific ligands such as antibodies or small molecules (transferrin, folate, and cyclic Arg-Gly-Asp) are conjugated to the liposomes by covalently coupling to the reactive phospholipids in the membrane or hydrophobically anchoring into the liposomal membrane [57, 58]. Furthermore, stimulus-responsive liposomes based on Stimulus-sensitive polymers or nanoparticles have recently been reported. These liposomes exhibit remote control of drug release by light irradiation or ultrasound [59, 60]. Recently, non-ionic surfactant-containing liposomes loaded with paclitaxel showed potential to overcome MDR through a combination of drug delivery, permeability glycoprotein (P-gp) inhibition, and ATP depletion [61]. P-gp is a drug efflux transporter over-expressed on MDR tumor cells.

2.1.2. Solid Lipid Nanoparticles

After preliminary efforts from the Speiser group [62], solid lipid nanoparticles (SLNs) have been studied as potential drug carriers due to various advantages, including the ability to incorporate lipophilic and hydrophilic compounds, enhanced colloidal stability, low cost, and a convenient mass production process [27, 28]. In contrast to emulsions and liposomes, the particle matrix of SLNs is composed of solid lipids such as triglyceride esters of hydrogenated fatty acids. In particular, SLNs can encapsulate a broad range of lipophilic chemotherapeutic agents. Xu *et al.* showed that docetaxel-loaded SLNs (approximately 120 nm) with an encapsulation efficiency >90% lead to a low burst effect within the first day and a sustained release for the next 29 days *in vivo* [63]. Yassin *et al.* prepared 5-FU-loaded SLNs by a double emulsion-solvent evaporation technique (w/o/w) to treat colon cancer [64]. On the other hand, Dong *et al.* prepared several different DOX- and Taxol-loaded lipid nanoparticles to overcome

Table 2. Various Nanoparticles for the Loading and Delivery of Chemotherapeutics

Category		Featured Structure	Advantages	References
Organic nanoparticle	Liposome	lipid bi-layered	biocompatible large amount drug loading	[25, 26]
	Solid lipid nanoparticle	stable compact lipid matrix	biocompatible hydrophobic drug loading	[27, 28]
	Micelles	micellized by amphiphilic compounds	hydrophobic drug loading	[29-32]
	Polymeric nanoparticles	synthetic polymer matrix	stable in biological medium sustained release	[33-35]
	Polymersomes	bi-layered by synthetic polymer	large amount drug loading multi-drug loading	[36-40]
	Dendrimers	end-functionalized dendric structure	multi-functionality	[41]
	Natural polymers	brush- or gel-type	biocompatible	[42-46]
Inorganic nanoparticle	Mesoporous silica nanoparticle	high porous structure	large amount drug loading	[47-49]
	Hollow silica nanoparticle	central cavity structure	large amount drug loading	[50, 51]
	Magnetic nanoparticle	cavity and porous structure	theragnostics	[52, 53]
	Carbon nanotubes	containing π-electrons	pH-sensitive drug release	[54, 55]

P-gp-mediated MDR. In this work, DOX nanoparticles showed 6- to 8-fold lower IC_{50} values in P-gp-overexpressing human cancer cells than those of free DOX due to overcoming of MDR *via* P-gp inhibition and ATP depletion [65].

2.1.3. Micelles

Micelles, defined as self-assembled collections of amphiphilic surfactant molecules, are turning out to be an important tool in cancer therapeutics [29-32]. In particular, micelles play a key role in anticancer drug delivery because of the ability to stably load minute particles and chemotherapeutic agents [66]. Recently, Kim *et al*. demonstrated that pH-sensitive DOX-loaded polymeric micelles composed of poly(histidine-co-phenylalanine)-b-poly(ethylene glycol) and poly(L-lactic acid)-b-PEG-folate successfully kill both wild-type and DOX -resistant ovarian MDR cancer cells through active internalization and accelerated DOX release triggered by the low endosomal pH [67].

2.1.4. Polymeric Nanoparticles

Colloidal nanoparticles based on synthesized biocompatible polymers can encapsulate a therapeutic agent within their polymeric matrix or adsorbed or conjugated onto the surface [33-35]. In particular, polymeric nanoparticles can be targeted to specific sites by surface modifications, which cause specific interactions with receptors expressed on target cancer cells. Fonseca *et al*. reported a polymeric paclitaxel delivery system to improve the therapeutic index of the drug and avoid the adverse effects of Cremophor® EL [34]. To achieve this goal, paclitaxel-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles were prepared by the interfacial deposition method (<200 nm). Yoo *et al*. demonstrated that the polymeric drug carrier system of DOX-PLGA increased the encapsulation and loading efficiency and maintained release at the tumor site [33]. The polymeric nanoparticles exhibited lower IC_{50} values compared to those of free DOX. Effective *in vivo* anti-tumor activity was achieved with a single injection of the drug-loaded polymeric nanoparticles. In addition, Dhar *et al*. demonstrated that the targeted delivery of cisplatin by using prostate-specific membrane antigen-targeted nanoparticles based on PLGA-PEG polymers is approximately an order of magnitude greater than that of free cisplatin [68].

2.1.5. Polymersomes

Polymersomes are self-assembled polymeric vesicles of a diverse array of synthetic amphiphilic block copolymers or ionic block copolymers [36-40]. In general, the structure of a polymersome is similar to that of a liposome in that it has a hydrophobic layer and a large hydrophilic reservoir. However, the bi-layer of synthetic polymers is more stable to shear stresses applied by the flow of a biological medium than that of lipid membranes[69, 70]. Therefore, polymersomes have been applied as drug and gene delivery carriers because of great colloidal stability, forgoing the large storage capability of liposomes. Li *et al*. reported stable polymersomes using poly(butadiene)-b-poly(ethylene oxide) that increased the solubility of paclitaxel due to the presence of the polymersome hydrophobic layer [71]. Sanson *et al*. demonstrated that polymersomes made by the self-assembly of amphiphilic biodegradable poly(trimethylene carbonate)-b-poly(L-glutamic acid) block copolymers permitted the controlled release of DOX [72]. These polymersomes exhibited high drug-loading quantities (up to 47% w/w) and successful release in acidic tumor environments (e.g., in endosomes or in solid tumors). Furthermore, polymersomes can load and deliver multiple therapeutic agents, such as two-species chemotherapeutic agents and combinations of drugs and genes, to cancer cells [73, 74]. On the other hand, modification of polymeric chains enables conjugating a targeting moiety to polymersomes for specific delivery to tumor cells. Upadhyay *et al*. demonstrated that synthesized block copolymers composed of a polypeptide segment and a polysaccharide moiety (hyaluronan-block-poly[γ-benzyl glutamate]) exhibited remarkably controlled release and functionality [75]. Due to the presence of hyaluronan, moreover, the chemotherapy of the CD44 glycoprotein-expressing C6 glioma tumor cell lines was possible. As described, polymersomes are thus effective tools for cancer chemotherapy due to several advantages: biocompatibility, biodegradability, high loading capability, stability in biological media, and sustained drug release. Therefore, polymersomes based on synthetic biocompatible polymers will be developed for effective clinical cancer chemo-therapy instead of liposomal formulations using phospholipids in the near future.

2.1.6. Dendrimers

Dendrimers are defined by macromolecular compounds that comprise a series of branches around an inner core, the size and shape of which can be modified for effective drug delivery [41]. Bhadra *et al*. presented a PEGylated 4.0G polyamidoamine dendrimer loaded with 5-FU by Michael addition and exhaustive amidation reactions [76]. PEGylation of dendrimers reduced drug

leakage and hemolytic toxicity and improved drug-loading capacity and stability in biological medium for effective drug administration.

2.1.7. Natural Polymers

Natural polymers like chitosan, dextran, hyaluronan, and gelatin have exhibited biocompatibility, low toxicity, and biodegradability. Thus, hydrogels based on natural polymers have been shown to provide sustained, local delivery of a variety of chemotherapeutic agents [42-46]. Ruel-Gariépy *et al*. demonstrated that a thermosensitive hydrogel loaded with paclitaxel inhibits the growth of cancer cells in mice with low toxicity [45]. Konishi *et al*. presented a chemically cross-linked gelatin drug carrier incorporating CDDP [77]. In particular, CDDP molecules immobilized in the gelatin hydrogel were released from the hydrogel when the hydrogel was degraded. On the other hand, high molecular weight hyaluronan exhibited targeted drug delivery potential [78]. The localized delivery of drug-loaded hyaluronan molecules to CD44-expressing cancer cells at the surgical resection site presents the potential to control tumor recurrence after removal of the tumor.

2.2. Silica Nanoparticles

In general, silica nanoparticles have potential as drug carriers because silica is inert, synthesis of these nanoparticles is simple, particle size can be easily controlled, and the surface of these nanoparticles can be modified in diverse ways [47-51]. Mainly, mesoporous silica nanoparticles (MSNs) and hollow silica nanoparticles have been used for loading and delivery of chemotherapeutic agents to tumor sites.

2.2.1. Mesoporous Silica Nanoparticles

Among a variety of inorganic nanomaterials, mesoporous silica nanoparticle (MSNs) have been attractive as a drug delivery system due to their large surface areas, tunable pore sizes, controllable particle sizes and shapes, and easy functionalization of surfaces [47]. Slowing *et al*. presented a series of MCM-41-type MSNs that were effectively endocytosed by cancer cells [48]. Rim *et al*. demonstrated smart MSNs modified by pore blockers using enzyme-mediated mineralization [49]. The inorganic pore blocker coatings of MSNs can hold the loaded DOX under extracellular conditions, and instant DOX release can be achieved within tumor cells by dissolution of the inorganic pore blocker.

2.2.2. Hollow Silica Nanoparticles

Template-mediated hollow silica can be synthesized with small size, large drug-loading capacity, and exhibit a convenience for surface modification. In particular, Cao *et al*. demonstrated the synthesis of porous hollow silica nanoparticles (60–70 nm) by using calcium carbonate as the inorganic template [50]. The prepared hollow silica nanoparticles exhibited a three-stage pattern and exhibited a delayed drug release effect. Yang *et al*. reported the synthesis of hollow silica nanoparticles as drug carriers using silica-coated magnetic assemblies, which are composed of a number of magnetic nanocrystals, as templates [51]. The core cavity was achieved by removing the magnetic nanocrystals with acids, and the surface was modified with amines to introduce positive surface charge and further PEGylated to increase solubility in aqueous medium. DOX was loaded into the hollow silica nanoparticles, and notable sustained drug release from these nanoparticles was demonstrated.

2.3. Magnetic Nanoparticles

Recently, novel magnetic drug carriers based on inorganic compounds have been reported. Cao *et al*. reported PEG-modified hierarchically magnetic hollow nanoparticles assembled by Fe_3O_4 or γ-Fe_2O_3 nanosheets [52]. The magnetic hollow nanoparticles have a high drug loading capacity and a favorable drug release property. Moreover, Shin *et al*. presented hollow manganese oxide nanoparticles as a solid reservoir for increase drug loading capacities [53]. In particular, their magnetic properties enabled a bi-functional medical system of diagnostic imaging and therapy.

2.4. Carbon Nanotubes

Carbon nanotubes, carbon cylinders composed of benzene rings, exhibit many unique intrinsic physical and chemical properties and may be useful as molecular transporters for drug delivery and potential new therapies [54]. In particular, Liu *et al*. demonstrated the potential of carbon nanotubes as DOX delivery carriers [79]. PEGylated carbon nanotubes allow for high degrees of stacking of DOX, leading to an ultrahigh loading capacity, and pH-sensitive release. Zhang *et al*. also demonstrated a targeted drug delivery system that is triggered by changes in pH based on carbon nanotubes loaded with DOX [55]. Folic acid has been tethered to carbon nanotubes as a targeting agent to selectively deliver DOX into specific cancer cells with much higher efficiency than free DOX.

3. THE USAGE OF NANOPLATFORM IN CANCER CHEMOTHERAPY

Recently, cancer has been defined as a group of multiple diseases, which are ascribed to complex oncogenic signaling and unusual cancer metabolism [80-83]. Moreover, biological barriers to successful treatment are presented by cancer cells and their microenvironments, such as heterogeneity and chemo-resistance. Thus, to overcome limitations of current chemotherapeutic agents, well-tailored nanoplatforms have been used to attain the following results: 1) increase the solubility of chemotherapeutic agents, 2) improve delivery efficiency to a tumor site, 3) achieve sustained and stimulus-response release of chemotherapeutic agents, 4) chemosensitize tumors with RNAi, and 5) simultaneously deliver multiple therapeutics. In the next section, we will review various therapeutic strategies for enhancing chemotherapeutic efficacy.

3.1. Targeted Delivery

To improve patient survival and quality of life by effectively chemosensitizing cancer cells, the following two strategies are essential [4]. The administrated chemotherapeutic agents should be delivered to target tumor sites by overcoming biological barriers without loss of activity. Subsequently, chemotherapeutic agents should successfully and specifically kill cancer cells without damage to surrounding normal cells. To achieve these strategies, nanoplatforms have been used as drug carriers to simultaneously increase the concentration of chemotherapeutic agents at the target site and avoid adverse effects. In particular, the vasculature in tumor tissues is very different from normal tissue due to the remarkable growth rate of cancerous cells and their angiogenic effect. Angiogenic blood vessels induced by cancer cells possess large gaps between endothelial cells, unlike normal blood vessels. Passive targeting, induced by the enhanced permeability and retention effect, enables the accumulation of a drug loaded onto nanoparticles at tumor sites due to differences between normal and tumor vasculature [84, 85]. However, drug delivery systems that exploit enhanced permeability and retention have intrinsic targeting specificity limitations. Thus, active targeting of nanoplatforms based on receptor recognition has recently become an attractive option [86]. For molecular recognition of cancer cells, biomarkers, especially surface receptors, such as folate receptor, Her2/neu, and EGFR, can be used as direct targets [19, 87, 88]. As homing moieties, monoclonal antibodies, peptides, and aptamers have been immobilized on surface-functionalized drug carriers loaded with chemotherapeutic agents to achieve specific delivery to tumor sites and chemosensitization after intravenous administration [18, 19, 89, 90].

3.2. Stimulus-responsive Delivery

It has been observed that tumors produce aberrant micro-environments that can be distinguished from those of normal tissues [91]. Thus, stimulus-responsive delivery of chemotherapeutic

agents using activatable nanomaterials, exploiting changes of physico-chemical properties of nanomaterials or the cleavage of a specific linker in the tumor microenvironment, has recently been studied. The environmental variations include pH and enzymes that can release chemotherapeutic agents from activatable nanoplatforms [92-94]. For example, Bae, Y. H. group exhibited pH-sensitive micelles loading DOX for the reversion of MDR. poly(L-histidine)-b-PEG-folate as a backbone was synthesized and histidine groups enabled to the loaded DOX release dependant to the proton concentration for bypass Pgp efflux pump [92]. On the other hand, Kopeček, J. group demonstrated that enzyme-specific degradable peptide sequence (GFLG) was introduced to HPMA polymers as drug carriers. The grafted DOX at HPMA backbone was released by cathepsin B generated under the tumor environment that enabled to effectively reduce tumor size [95].

3.3. Multi-therapeutics Delivery

Although various chemotherapeutic agents have been developed, a variety of chemoresistance mechanisms, such as upregulation of anti-apoptotic genes, increased DNA damage repair, inhibition of apoptosis, increased drug efflux, and decreased drug influx, represent critical problems for effective cancer treatment without side effects [96-100]. Thus, systemic chemosensitization of cancer cells by the combination of therapeutic agents with different mechanisms of action to overcome chemoresistance is necessary for additive or synergistic cancer therapy [13, 101-104]. In this section, we will review several chemosensitization methods using delivery of therapeutics of multiple types.

3.3.1. RNAi for Chemosensitization

In 1998, Andrew Fire, Craig Mello, and colleagues discovered RNA interference (RNAi) [105]. In general, RNAi involves the silencing of endogenous gene expression by double-stranded RNA molecules *via* catalytic degradation of target mRNA. In Table **3**, several RNAi targets for chemosensitization and combination chemotherapeutic agents are presented according to their mode of action: 1) down-regulation of anti-apoptosis proteins, 2) inhibition of growth factor signaling and 3) oncogene signaling, and 4)

Table 3. Various RNAi Strategies for Chemosensitization

Target		Silencing Moiety	Chemo	Cancer Type	Ref.
Apoptotic root	Bcl-2	siRNA	5-FU/S-1	colorectal cancer	[108]
	Bcl-2	siRNA	Taxol	glioblastoma	[109]
	Bcl-2	siRNA	Taxol	breast cancer	[110]
	Bcl-2	siRNA	DOX	hepatoma	[111]
	Bcl-2	siRNA	DOX	ovarian cancer	[112]
	Bcl-xL	shRNA	DOX	prostate cancer	[18]
	survivin	Antisense oligonucleotide/ siRNA	CDDP/GEM	bladder cancer	[113]
Growth factor	EGFR	Antisense oligonucleotide	5-FU	glioblastoma	[114]
	VEGFR	siRNA	DOX	hepatoma	[115]
	VEGFR	siRNA	Taxol	breast	[116]
	IGF1R/EGFR	siRNA	DOX	hepatoma	[117]
Oncogene signaling	RET	siRNA	irinotecan	medullary tyroid carcinoma	[119]
	Gli-1	siRNA	5-FU	hepatoma	[120]
	Akt	siRNA	Hydroxycamptothecin	lung cancer	[121]
	Nek2	siRNA	CDDP	colorectal cancer	[122]
	Rad51	siRNA	CDDP	hela	[123]
	hTERT	siRNA	DOX	breast cancer	[124]
	Nrf2	shRNA	CDDP/Taxol/DOX/5-FU	cerical cancer	[125]
MDR	P-gp	siRNA	DOX	breast cancer	[90]
	MRP1, Bcl2	Oligonucleotide	DOX	lung cancer	[126]
	MDR1/MRP1, Bcl2	siRNA	DOX	lung cancer	[127]
	mdr1a/mdr1b	siRNA	vinblastine	lymphosarcoma	[128]
	miR-27a	Antagomir	VCR/DOX/5-FU/CDDP	gastric cancer	[129]
	miR326	siRNA	VP-16/DOX	breast cancer	[130]
	miR200c	siRNA	CDDP	gastric cancer	[131]

Index; 5-FU: 5-, Taxol: paclitaxel, DOX: doxorubicin, CDDP: cisplatin, GEM: gemcitabine, VCR: vincristin

reversion of MDR. In particular, functional nanoplatforms have recently been developed for RNAi to minimize unwanted side effects and overcome biological hurdles to therapy [106, 107].

Among them, down-regulation of apoptosis-related proteins, such as Bcl-2, Bcl-xL, survivin, and p53, represents a successful strategy for the chemosensitization of cancer cells [18, 108-113]. For example, Wang *et al*. knocked down Bcl-2 expression using small interfering RNA (siRNA) loaded onto a synthetic co-polymer as a non-viral vector to chemosensitize against Taxol and induce apoptosis in breast cancer cells [110]. Cao *et al*. presented hierarchical cationic nano-micelles for loading of Bcl-2 siRNA and DOX [111]. Additionally, Chen *et al*. presented mesoporous silica nanoparticles for co-delivery of DOX and Bcl-2 siRNA [112]. These nanoplatforms simultaneously delivered chemotherapeutic agents and Bcl-2 family-targeted siRNA into target cancer cells, and the Bcl-2 family siRNA effectively decreased cell viability of cancer cells *via* a synergistic effect.

On the other hand, the specific inhibition of growth factor [114-118] and oncogenic signaling [119-125] with RNAi has demonstrated therapeutic effects against cancer cells. Zhu *et al*. revealed biodegradable cationic micelles using tri-block copolymers and applied them to the delivery of VEGF siRNA and Taxol into cancer cells [116]. Furthermore, Dickerson *et al*. reported that targeted delivery of epidermal growth factor receptor (EGFR) siRNAs by nanogels may be a promising strategy to increase the efficacy of chemotherapy drugs for the treatment of ovarian cancer [118].

Another way to augment chemotherapeutic efficacy is by reversion of MDR. Reversion of MDR is essential for increased chemotherapeutic agent influx by downregulating P-gp (MDR1), MRP1, and microRNAs related to MDR [90, 126-131]. Jiang *et al*. demonstrated a sequential treatment strategy with RGD-modified liposomes containing P-gp-targeted siRNA to reverse drug resistance and DOX [90]. Moreover, simultaneous co-delivery of DOX and siRNA (or antisense oligonucleotides) targeted to MRP1 mRNA in the liposomal drug delivery system were reported to inhibit drug resistance conferred by MRP1, the drug efflux pump [126, 127].

3.3.2. Combination Chemotherapy using Multiple Agents

To increase tumor regression rates relative to the individual drugs, recent clinical studies have studied combination therapy regimens using multiple drugs with different mechanisms. Kim *et al*. reported that combination chemotherapy with oxaliplatin, 5-FU, and folinic acid is an active and well-tolerated regimen as a first-line treatment in patients with metastatic or recurrent gastric cancer [132]. Ahmed *et al*. demonstrated that combination therapy using polymersomes loaded with DOX and Taxol exhibits distinct solubility characteristics for the treatment of aggressive metastatic breast tumors [73]. In particular, Sengupta *et al*. demonstrated multidrug-loadable nanocells, with DOX as a chemotherapeutic agent conjugated to the nanoparticle and combretastatin as an anti-angiogenesis agent trapped within the lipid envelope. The fabricated nanocells can be specifically targeted to tumor vasculature by recognizing specific molecular signatures on the vasculature. Thus, the nanocell concept represents a significant advance in cancer therapy *via* simultaneous anti-angiogenic effect and induction of apoptosis of cancer cells [133].

3.4. Theragnosis using Nanoplatforms

As described in previous sections, various nanoplatform carriers for chemotherapeutic agents have been developed. Despite these nanomedicines developments, however, it is still difficult to monitor the efficiency of drug delivery to the tumor site and determine the next dosing time point for optimal cancer chemotherapy. Thus, several researchers have recently demonstrated theragnosis of cancer based on molecular imaging [19, 93, 134-139]. Here, theragnosis means simultaneous diagnosis and therapy of cancer using nanocomposites containing diagnostics and therapeutics. To achieve successful theragnosis in oncology, a combination of therapeutic agent and imaging probe in one nanoparticle is necessary. Furthermore, the theragnostic nanoplatforms, containing therapeutic agents and imaging probes, should be modified with a proper targeting moiety (e.g., an antibody) for the specific delivery to target cancer cells. Namely, the following key elements should be satisfied; 1) well-tailored nanostructures loaded with a chemotherapeutic agent and an imaging probe, 2) precise recognition of the release profile of the loaded chemo-therapeutic agent from the nanostructure, 3) targeted delivery of the nanostructure specifically to cancer cells, and 4) imaging and monitoring the presence of the nanostructure at the tumor site. With the aid of theragnosis using functional nanoplatforms, cancer treatment may become shorter, safe, and more efficient. In this section, several theragnostic strategies using functional nanoparticles as the carriers of diagnostic and therapeutic agents are reviewed.

Recently, the Kwon group reported remarkable theragnostic nanoplatforms that deliver a near-infrared fluorescent probe for *in vivo* imaging and a chemotherapeutic agent for cancer treatment, simultaneously [140, 141]. These nanoplatforms accumulated at the tumor site by fast cellular uptake for the enhancement of therapeutic efficacy. Cy5.5 is a near-infrared fluorescent probe used to enable non-invasive *in vivo* imaging of tumor-bearing animals because the body is "transparent" in the near-infrared window (650 to 900 nm) [88, 142]. In particular, the optimized treatment protocol was shown to reduce tumor size without the severe toxicity associated with free Taxol administration.

On the other hand, Yang *et al*. recently reported a proof of concept for theragnosis based on high-resolution magnetic resonance imaging (MRI) imaging [19, 134]. Several magnetic nanoparticles and DOX enveloped by an amphiphilic surfactant were formulated by a nanoemulsion method. Her2/neu-targeted, antibody-conjugated theragnostic nanoplatforms were monitored at the tumor site by T2-weighted MRI, and the reduced tumor growth caused by the released DOX was observed. In addition, the same group recently demonstrated an advanced theragnostic nanoplatform for the determination of optimal dosing timing *via* MRI [93]. In this report, the property was explained with one term, "self-documenting." The smart nanoplatform was composed of anti HER2/neu antibody-modified, pH-sensitive, drug-delivering magnetic nanoparticles. In particular, DOX molecules were loaded onto pyrene molecules, due to strong π–π interactions, for pH-sensitive release within the cell. As expected, the loaded magnetic nanoparticles enabled the observation by MRI of HER2/neu-expressing cancer cells *in vivo* and the release of DOX at the tumor site. As reviewed in this section, functional nanoplatforms have been developed as theragnostic nano-drugs based on molecular imaging that would shed light on optimized personalized cancer therapy (i.e., the enhancement of therapeutic efficacy without adverse effects).

PROSPECT AND CLINICAL TRIAL

The major drawback of chemotherapeutics stems from adverse effects of non-specific toxicities on normal tissues and the emergence of drug resistance. Functional nanoplatforms with diverse species of nanoscale constructs discussed above will provide solutions to overcome the problems of current chemotherapeutics. Enhancement of chemotherapeutic indices by widespread use of functional nanoplatforms will offer a unique opportunity to personalize cancer therapy, providing solutions to the problems of optimizing the therapeutic index and drug resistance associated with conventional but highly potent chemotherapeutic agents. Furthermore, nanoscale imaging technology combined with the nanoscale drug delivery systems will

allow the development of functional nanoplatforms to achieve "smart" theragnostic individualization of cancer treatment in the coming years. These include detecting malignant cells with active targeting moieties, visualizing the precise location of the tumor (i.e., *in vivo* imaging), eliminating cancer cells while avoiding adverse effects (e.g., active targeting and controlled release), and monitoring treatment effects in real time, which will minimize the emergence of resistance as well as permit early drug resistance surveillance. Together, the functional nanoplatforms will ultimately reshape the future of anti-cancer therapy by enhancing the chemotherapeutic index and tailoring treatment for a diverse spectrum of cancer patients.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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