# Therapeutic Nanoparticles for Drug Delivery in Cancer

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**Abstract** Cancer nanotherapeutics are rapidly progressing and are being implemented to solve several limitations of conventional drug delivery systems such as nonspecific biodistribution and targeting, lack of water solubility, poor oral bioavailability, and low therapeutic indices. To improve the biodistribution of cancer drugs, nanoparticles have been designed for optimal size and surface characteristics to increase their circulation time in the bloodstream. They are also able to carry their loaded active drugs to cancer cells by selectively using the unique pathophysiology of tumors, such as their enhanced permeability and retention effect and the tumor microenvironment. In addition to this passive targeting mechanism, active targeting strategies using ligands or antibodies directed against selected tumor targets amplify the specificity of these therapeutic nanoparticles. Drug resistance, another obstacle that impedes the efficacy of both molecularly targeted and conventional chemotherapeutic agents, might also be overcome, or at least reduced, using nanoparticles. Nanoparticles have the ability to accumulate in cells without being recognized by P-glycoprotein, one of the main mediators of multidrug resistance, resulting in the increased intracellular concentration of drugs. Multifunctional and multiplex nanoparticles are now being actively investigated and are on the horizon as the next generation of nanoparticles, facilitating personalized and tailored cancer treatment.

Conventional chemotherapeutic agents are distributed nonspecifically in the body where they affect both cancerous and normal cells, thereby limiting the dose achievable within the tumor and also resulting in suboptimal treatment due to excessive toxicities. Molecularly targeted therapy has emerged as one approach to overcome the lack of specificity of conventional chemotherapeutic agents (1). However, the development of resistance in cancer cells can evade the cytotoxicity not only of conventional chemotherapeutics but also of these newer molecularly targeted therapeutics (2).

Nanoparticles, by using both passive and active targeting strategies, can enhance the intracellular concentration of drugs in cancer cells while avoiding toxicity in normal cells (3, 4). Furthermore, when nanoparticles bind to specific receptors and then enter the cell, they are usually enveloped by endosomes via receptor-mediated endocytosis, thereby bypassing the recognition of P-glycoprotein, one of the main drug resistance mechanisms (5). However, although nanoparticles offer many advantages as drug carrier systems, there are still many limitations to be solved such as poor oral bioavailability, instability in circulation, inadequate tissue distribution, and toxicity.

In this review, we will address, first, the types and characteristics of nanoparticles; second, how nanoparticles are being used as drug delivery systems to kill cancer cells more effectively and also to reduce or overcome drug resistance; and third, how nanoparticles will be developed to improve their therapeutic efficacy and functionality in future cancer treatments.

## Types of Nanoparticles Used as Drug Delivery Systems

Nanoparticles applied as drug delivery systems are submicron-sized particles (3-200 nm), devices, or systems that can be made using a variety of materials including polymers (polymeric nanoparticles, micelles, or dendrimers), lipids (liposomes), viruses (viral nanoparticles), and even organometallic compound (nanotubes; Table 1).

### Polymer-based drug carriers

Depending on the method of preparation, the drug is either physically entrapped in or covalently bound to the polymer matrix (6). The resulting compounds may have the structure of capsules (polymeric nanoparticles), amphiphilic core/shell (polymeric micelles), or hyperbranched macromolecules (dendrimers; Fig. 1). Polymers used as drug conjugates can be divided into two groups of natural and synthetic polymers.

***Polymeric nanoparticles (polymer-drug conjugates).*** Polymers such as albumin, chitosan, and heparin occur naturally and have been a material of choice for the delivery of oligonucleotides, DNA, and protein, as well as drugs. Recently, a nanoparticle formulation of paclitaxel, in which serum

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**Table 1.** Types of nanocarriers for drug delivery

| System | Structure | Characteristics | Examples of compounds | Ref. |
|---|---|---|---|---|
| Polymeric nanoparticles (polymer-drug conjugates) | Drugs are conjugated to the side chain of a linear polymer with a linker (cleavable bond) | (*a*) Water-soluble, nontoxic, biodegradable<br>(*b*) Surface modification (pegylation)<br>(*c*) Selective accumulation and retention in tumor tissue (EPR effect)<br>(*d*) Specific targeting of cancer cells while sparing normal cells—receptor-mediated targeting with a ligand | Albumin-Taxol (Abraxane)<br>PGA-Taxol (Xyotax)<br>PGA-Camptothecin (CT-2106)<br>HPMA-DOX (PK1)<br>HPMA-DOX-galactosamine (PK2) | (7)<br>(11)<br>(12)<br>(14)<br>(58) |
| Polymeric micelles | Amphiphilic block copolymers assemble and form a micelle with a hydrophobic core and hydrophilic shell | (*a*) Suitable carrier for water-insoluble drug<br>(*b*) Biocompatible, self-assembling, biodegradable<br>(*c*) Ease of functional modification<br>(*d*) Targeting potential | PEG-pluronic-DOX<br>PEG-PAA-DOX (NK911)<br>PEG-PLA-Taxol (Genexol-PM) | (16)<br>(17)<br>(18) |
| Dendrimers | Radially emerging hyperbranched synthetic polymer with regular pattern and repeated units | (*a*) Biodistribution and PK can be tuned<br>(*b*) High structural and chemical homogeneity<br>(*c*) Ease of functionalization, high ligand density<br>(*d*) Controlled degradation<br>(e) Multifunctionality | PAMAM-MTX<br>PAMAM-platinate | (64)<br>(21) |
| Liposomes | Self-assembling closed colloidal structures composed of lipid bilayers | (*a*) Amphiphilic, biocompatible<br>(*b*) Ease of modification<br>(*c*) Targeting potential | Pegylated liposomal DOX (Doxil)<br>Non-pegylated liposomal DOX (Myocet)<br>Liposomal daunorubicin (DaunoXome) | (22)<br>(23)<br>(24) |
| Viral nanoparticles | Protein cages, which are multivalent, self-assembled structures | (*a*) Surface modification by mutagenesis or bioconjugation—multivalency<br>(*b*) Specific tumor targeting, multifunctionality<br>(*c*) Defined geometry and remarkable uniformity<br>(*d*) Biological compatibility and inert nature | HSP-DOX<br>CPMV-DOX | (29, 30)<br>(27) |
| Carbon nanotubes | Carbon cylinders composed of benzene ring | (*a*) Water-soluble and biocompatible through chemical modification (organic functionalization)<br>(*b*) Multifunctionality | CNT-MTX<br>CNT-amphotericin B | (34)<br>(33) |

Abbreviations: PGA, poly-(L-glutamate); HPMA, *N*-(2-hydroxypropyl)-methacrylamide copolymer; PEG, polyethylene glycol; PAA, poly-(L-aspartate); PLA, poly-(L-lactide); PAMAM, poly(amidoamine); DOX, doxorubicin; MTX, methotrexate; PK, pharmacokinetics; EPR, enhanced permeability and retention; CNT, carbon nanotube; HSP, heat shock protein; CPMV, cowpea mosaic virus.

albumin is included as a carrier [nanometer-sized albumin-bound paclitaxel (Abraxane); Fig. 1A], has been applied in the clinic for the treatment of metastatic breast cancer (7). Besides metastatic breast cancer, Abraxane has also been evaluated in clinical trials involving many other cancers including non–small-cell lung cancer (phase II trial) and advanced nonhematologic malignancies (phase I and pharmacokinetics trials; refs. 8, 9).

Among synthetic polymers such as *N*-(2-hydroxypropyl)-methacrylamide copolymer (HPMA), polystyrene-maleic anhydride copolymer, polyethylene glycol (PEG), and poly-L-glutamic acid (PGA), PGA was the first biodegradable polymer to be used for conjugate synthesis (10). Several representative chemotherapeutics that are used widely in the clinic have been tested as conjugates with PGA *in vitro* and *in vivo* and showed encouraging abilities to circumvent the shortcomings of their

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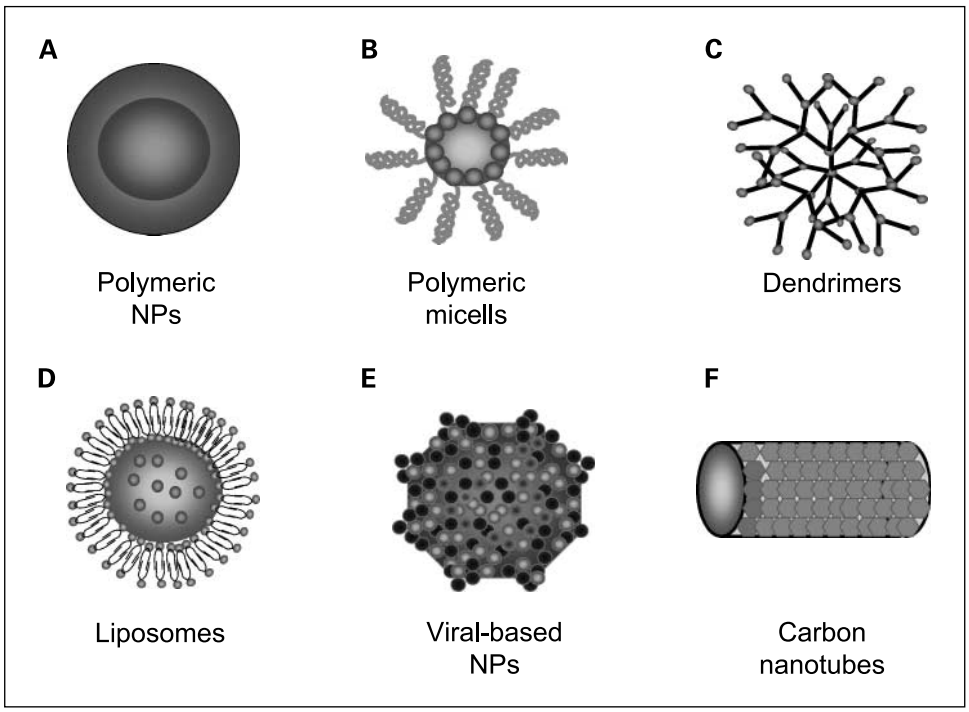


**Fig. 1.** Types of nanocarriers for drug delivery. *A,* polymeric nanoparticles: polymeric nanoparticles in which drugs are conjugated to or encapsulated in polymers. *B,* polymeric micelles: amphiphilic block copolymers that form to nanosized core/shell structure in aqueous solution. The hydrophobic core region serves as a reservoir for hydrophobic drugs, whereas hydrophilic shell region stabilizes the hydrophobic core and renders the polymer to be water-soluble. *C,* dendrimers: synthetic polymeric macromolecule of nanometer dimensions, which is composed of multiple highly branched monomers that emerge radially from the central core. *D,* liposomes: self-assembling structures composed of lipid bilayers in which an aqueous volume is entirely enclosed by a membranous lipid bilayer. *E,* viral-based nanoparticles: in general structure are the protein cages, which are multivalent, self-assembles structures. *F,* carbon nanotubes: carbon cylinders composed of benzene rings.

free drug counterparts (10). Among them, Xyotax (PGA-paclitaxel; ref. 11) and CT-2106 (PGA-camptothecin; ref. 12) are now in clinical trials.

HPMA and PEG are the most widely used nonbiodegradable synthetic polymers (13). PK1, which is a conjugate of HPMA with doxorubicin, was the synthetic polymer-drug conjugate to be evaluated in clinical trials as an anticancer agent. A phase I clinical trial has been completed in patients with a variety of tumors that were refractory or resistant to prior therapy such as chemotherapy and/or radiation (14). PK1 should be further evaluated in the next level of clinical trials.

***Polymeric micelles (amphiphilic block copolymers).*** The functional properties of micelles are based on amphiphilic block copolymers, which assemble to form a nanosized core/shell structure in aqueous media (Fig. 1B). The hydrophobic core region serves as a reservoir for hydrophobic drugs, whereas the hydrophilic shell region stabilizes the hydrophobic core and renders the polymers water-soluble, making the particle an appropriate candidate for i.v. administration (15). The drug can be loaded into a polymeric micelle in two ways: physical encapsulation (16) or chemical covalent attachment (17).

The first polymeric micelle formulation of paclitaxel, Genexol-PM (PEG-poly(D,L-lactide)-paclitaxel), is a cremophor-free polymeric micelle-formulated paclitaxel. A phase I and pharmacokinetic study has been conducted in patients with advanced refractory malignancies (18). Multifunctional polymeric micelles containing targeting ligands and imaging and therapeutic agents are being actively developed (19) and will become the mainstream among several models of the micellar formulation in the near future.

***Dendrimers.*** A dendrimer is a synthetic polymeric macromolecule of nanometer dimensions, composed of multiple highly branched monomers that emerge radially from the central core (Fig. 1C). Properties associated with these dendrimers such as their monodisperse size, modifiable surface functionality, multivalency, water solubility, and available internal cavity make them attractive for drug delivery (20).

Polyamidoamine dendrimer, the dendrimer most widely used as a scaffold, was conjugated with cisplatin (21). The easily modifiable surface characteristic of dendrimers enables them to be simultaneously conjugated with several molecules such as imaging contrast agents, targeting ligands, or therapeutic drugs, yielding a dendrimer-based multifunctional drug delivery system (20).

### Lipid-based drug carriers

***Liposomes.*** Liposomes are self-assembling closed colloidal structures composed of lipid bilayers and have a spherical shape in which an outer lipid bilayer surrounds a central aqueous space (Fig. 1D). Currently, several kinds of cancer drugs have been applied to this lipid-based system using a variety of preparation methods. Among them, liposomal formulations of the anthracyclines doxorubicin (Doxil, Myocet) and daunorubicin (DaunoXome) are approved for the treatment of metastatic breast cancer and AIDS-related Kaposi's sarcoma (22–24). Besides these approved agents, many liposomal chemotherapeutics are currently being evaluated in clinical trials (25). The next generation of liposomal drugs may be immunoliposomes, which selectively deliver the drug to the desired sites of action (26).

### Viral nanoparticles

A variety of viruses including cowpea mosaic virus, cowpea chlorotic mottle virus, canine parvovirus, and bacteriophages have been developed for biomedical and nanotechnology applications that include tissue targeting and drug delivery (Fig. 1E). A number of targeting molecules and peptides can be displayed in a biologically functional form on their capsid surface using chemical or genetic means. Therefore, several ligands or antibodies including transferrin, folic acid, and

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single-chain antibodies have been conjugated to viruses for specific tumor targeting *in vivo* (27). Besides this artificial targeting, a subset of viruses, such as canine parvovirus, have natural affinity for receptors such as transferrin receptors that are up-regulated on a variety of tumor cells (28). By targeting heat shock protein, a dual-function protein cage with specific targeting and doxorubicin encapsulation has been developed (29, 30).

**Carbon nanotubes**

Carbon nanotubes are carbon cylinders composed of benzene rings (Fig. 1F) that have been applied in biology as sensors for detecting DNA and protein, diagnostic devices for the discrimination of different proteins from serum samples, and carriers to deliver vaccine or protein (31). Carbon nanotubes are completely insoluble in all solvents, generating some health concerns and toxicity problems. However, the introduction of chemical modification to carbon nanotubes can render them water-soluble and functionalized so that they can be linked to a wide variety of active molecules such as peptides, proteins, nucleic acids, and therapeutic agents (32).

Antifungal agents (amphotericin B) or anticancer drugs (methotrexate) have been covalently linked to carbon nanotubes with a fluorescent agent (FITC). In an *in vitro* study, drugs bound to carbon nanotubes were shown to be more effectively internalized into cells compared with free drug alone and to have potent antifungal activity (33, 34). The multiple covalent functionalizations on the sidewall or tips of carbon nanotubes allows them to carry several molecules at once, and this strategy provides a fundamental advantage in the treatment of cancer.

## Targeted Delivery of Nanoparticles

Ideally, for anticancer drugs to be effective in cancer treatment, they should first, after administration, be able to reach the desired tumor tissues through the penetration of barriers in the body with minimal loss of their volume or activity in the blood circulation. Second, after reaching the tumor tissue, drugs should have the ability to selectively kill tumor cells without affecting normal cells with a controlled release mechanism of the active form. These two basic strategies are also associated with improvements in patient survival and quality of life by increasing the intracellular concentration of drugs and reducing dose-limiting toxicities simultaneously. Increasingly, nanoparticles seem to have the potential to satisfy both of these requirements for effective drug carrier systems.

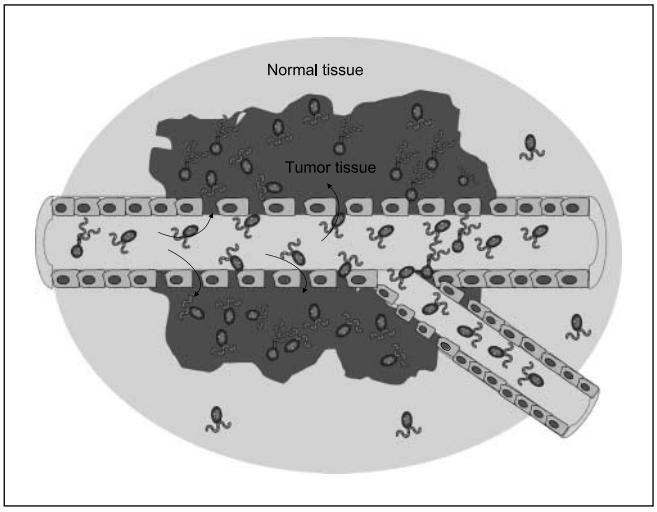


**Fig. 2.** Tumor targeting of nanoparticles passively by enhanced permeability and retention. Long-circulating therapeutic nanoparticles accumulate passively in solid tumor tissue by the enhanced permeability and retention effect. Angiogenic tumor vessels are disorganized and leaky. Hyperpermeable angiogenic tumor vasculature allows preferential extravasation of circulating nanoparticles.

## Size and Surface Characteristics of Nanoparticles

To effectively deliver drug to the targeted tumor tissue, nanoparticles must have the ability to remain in the bloodstream for a considerable time without being eliminated. Conventional surface nonmodified nanoparticles are usually caught in the circulation by the reticuloendothelial system, such as the liver and the spleen, depending on their size and surface characteristics (35). The fate of injected nanoparticles can be controlled by adjusting their size and surface characteristics.

***Size.*** One of the advantages of nanoparticles is that their size is tunable. The size of nanoparticles used in a drug delivery system should be large enough to prevent their rapid leakage into blood capillaries but small enough to escape capture by fixed macrophages that are lodged in the reticuloendothelial system, such as the liver and spleen. The size of the sinusoid in the spleen and fenestra of the Kuffer cells in the liver varies from 150 to 200 nm (36) and the size of gap junction between endothelial cells of the leaky tumor vasculature may vary from 100 to 600 nm (37). Consequently, the size of nanoparticles should be up to 100 nm to reach tumor tissues by passing through these two particular vascular structures.

***Surface characteristics.*** In addition to their size, the surface characteristics of nanoparticles are also an important factor determining their life span and fate during circulation relating to their capture by macrophages. Nanoparticles should ideally have a hydrophilic surface to escape macrophage capture (38). This can be achieved in two ways: coating the surface of nanoparticles with a hydrophilic polymer, such as PEG, protects them from opsonization by repelling plasma proteins; alternatively, nanoparticles can be formed from block copolymers with hydrophilic and hydrophobic domains (15, 39).

## Passive Targeting by Nanoparticles

***Enhanced permeability and retention effect.*** Nanoparticles that satisfy the size and surface characteristics requirements described above for escaping reticuloendothelial system capture have the ability to circulate for longer times in the bloodstream and a greater chance of reaching the targeted tumor tissues. The unique pathophysiologic characteristics of tumor vessels enable macromolecules, including nanoparticles, to selectively accumulate in tumor tissues (3). Fast-growing cancer cells demand the recruitment of new vessels (neovascularization) or rerouting of existing vessels near the tumor mass to supply them with oxygen and nutrients (40). The resulting imbalance of angiogenic regulators such as growth factors and matrix metalloproteinases makes tumor vessels highly disorganized and dilated with numerous pores showing enlarged gap junctions between endothelial cells and compromised lymphatic drainage (40). These features are called the enhanced permeability and retention effect, which constitutes an important mechanism

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by which macromolecules, including nanoparticles, with a molecular weight above 50 kDa, can selectively accumulate in the tumor interstitium (Fig. 2; ref. 3).

***Tumor microenvironment.*** Another contributor to passive targeting is the unique microenvironment surrounding tumor cells, which is different from that of normal cells. Fast-growing, hyperproliferative cancer cells show a high metabolic rate, and the supply of oxygen and nutrients is usually not sufficient for them to maintain this. Therefore, tumor cells use glycolysis to obtain extra energy, resulting in an acidic environment (41). The pH-sensitive liposomes are designed to be stable at a physiologic pH of 7.4, but degraded to release active drug in target tissues in which the pH is less than physiologic values, such as in the acidic environment of tumor cells (42).

Additionally, cancer cells express and release unique enzymes such as matrix metalloproteinases, which are implicated in their movement and survival mechanisms (43). An albumin-bound form of doxorubicin incorporating a matrix metalloproteinase-2–specific octapeptide sequence between the drug and the carrier was observed to be efficiently and specifically cleaved by matrix metalloproteinase-2 in an *in vitro* study (44).

## Active Targeting by Nanoparticles

A drug delivery system comprising a binary conjugate (i.e., polymer-drug conjugate) that depends only on passive targeting mechanisms inevitably faces intrinsic limitations to its specificity. One approach suggested to overcome these limitations is the inclusion of a targeting ligand or antibody in polymer-drug conjugates (4). Initially, direct conjugation of an antibody to a drug was attempted. However, in clinical trials conducted thus far, such early antibody-drug conjugates have failed to show superiority as a targeted delivery tool for the treatment of cancer (45). One of the reasons for this is that the number of drug molecules that can be loaded on the antibody while preserving its immune recognition is limited.

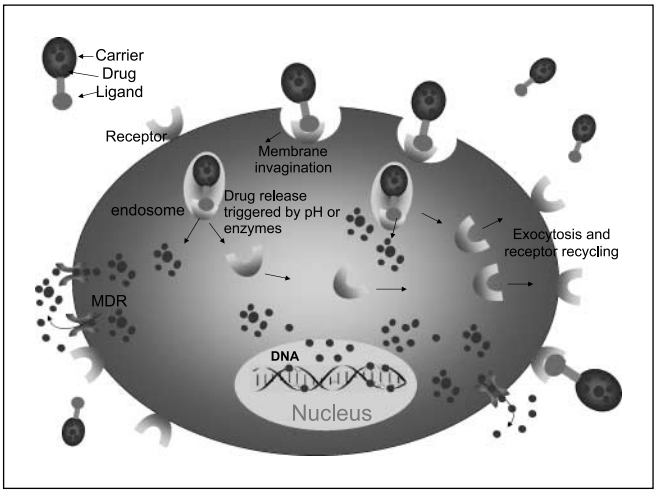


**Fig. 3.** Internalization of nanoparticles via receptor-mediated endocytosis. Tumor-specific ligands or antibodies on the nanoparticles bind to cell-surface receptors, which trigger internalization of the nanoparticles into the cell through endosome. As a pH value in the interior of the endosome becomes acidic, the drug is released from the nanoparticles and goes into the cytoplasm. Drug-loaded nanoparticles bypass the P-glycoprotein efflux pump not being recognized when the drug enters cells, leading to high intracellular concentration.

The recent development and introduction of a wide variety of liposomes and polymers as drug delivery carriers increases the potential number of drugs that can be conjugated to targeted nanoparticles without compromising their targeting affinity relative to earlier antibody-drug conjugates. Taking advantage of this array of carriers, targeting moieties, and drugs, many recently developed active targeting drug conjugates use a ternary structure composed of a ligand or antibody as a targeting moiety, a polymer or lipid as a carrier, and an active chemotherapeutic drug. When constructing ternary structure nanoparticles, some factors must be considered to create more efficient delivery systems.

***Antigen or receptor expression.*** Ideally, cell-surface antigens and receptors should have several properties that render them particularly suitable tumor-specific targets (4). First, they should be expressed exclusively on tumor cells and not expressed on normal cells. Second, they should be expressed homogeneously on all targeted tumor cells. Last, cell-surface antigens and receptors should not be shed into the blood circulation.

***Internalization of targeted conjugates.*** Whether targeted conjugates can be internalized after binding to target cells is an important criterion in the selection of proper targeting ligands.

Internalization usually occurs via receptor-mediated endocytosis (Fig. 3). Using the example of the folate receptor, when a folate-targeted conjugate binds with folate receptor on the cell surface, the invaginating plasma membrane envelopes the complex of the receptor and ligand to form an endosome. Newly formed endosomes are transferred to target organelles. As the pH value in the interior of the endosome becomes acidic and lysozymes are activated, the drug is released from the conjugate and enters the cytoplasm, provided the drug has the proper physico-chemical properties to cross the endosomal membrane. Released drugs are then trafficked by their target organelle depending on the drug. Meanwhile, the folate receptor released from the conjugate returns to the cell membrane to start a second round of transport by binding with new folate-targeted conjugates (46).

## Therapeutic Application of Ligand-Targeted Nanoparticles

The folate receptor is a well-known tumor marker that binds vitamin folate and folate-drug conjugates with a high affinity and carries these bound molecules into the cells via receptor-mediated endocytosis (46). We have checked the incidence of folate receptor expression in human head and neck primary and metastatic tumor tissues and compared them with normal tissues such as the bone marrow. Folate receptor expression was found in 53% of these tumor samples whereas normal bone marrow cells did not show any folate receptor expression (47). Recently, we generated a new folate receptor–targeted nanoparticle formulation of paclitaxel using heparin as a carrier [heparin-folate-Taxol (paclitaxel), HFT] and tested it using nude mouse animal models. This novel ternary nanoparticle HFT showed more potent activity against the growth of tumor xenografts of human KB and paclitaxel-resistant KB derivatives than did binary heparin-Taxol or free drug (paclitaxel; ref. 48).

Aptamers are oligonucleic acids such as DNA or RNA that bear unique three-dimensional conformations capable of binding to target antigens with high affinity and specificity (49). They have been applied to drug delivery systems as a ligand to enhance selectivity (50). The *in vivo* efficacy of

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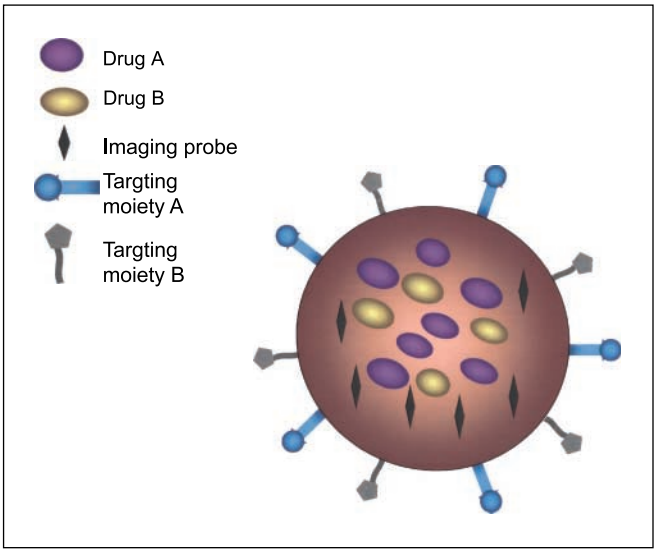


**Fig. 4.** Multifunctional nanoparticle. The following are illustrated: the ability to carry one or more therapeutic agents; biomolecular targeting through one or more conjugated antibodies or other recognition agents; imaging signal amplification, by way of co-encapsulated contrast agents. These nanoparticles will eventually be capable of detecting malignant cells (active targeting moiety), visualizing their location in the body (real-time *in vivo* imaging), killing the cancer cells without side effects by saving normal cells (active targeting and controlled drug released system or photothermal ablation), and monitoring the treatment effect in real time.

docetaxel-encapsulated poly(lactic-co-glycolic acid) nanoparticle conjugated with an aptamer to target prostate-specific membrane antigens was evaluated using an animal model (51).

Transferrin, a serum glycoprotein, works as a transporter to deliver iron through the blood and into cells by binding to the transferrin receptor and subsequently being internalized via receptor-mediated endocytosis (52). Because the transferrin receptor is overexpressed in tumor tissues compared with normal tissues, it has been investigated as a target for tumor-specific drug delivery (53). Transferrin-conjugated paclitaxel-loaded [poly(lactic-co-glycolic acid) polymer] nanoparticles displayed greater inhibitory effects on cell growth than free paclitaxel in MCF-7 and MCF-7/Adr cells (54). Transferrin was also conjugated to liposomes to increase the transfection efficacy of p53, resulting in the sensitization of the transfected cancer cells/xenografts to ionizing radiation (55).

Lectins are proteins that recognize and bind to carbohydrate moieties attached to protein molecules (glycans) on the extracellular side of the plasma membrane. Cancer cells often express different glycans compared with their normal counterparts. Therefore, lectins could be used as targeting molecules to direct drugs specifically to desired cells and tissues (56). This protein (lectin)-carbohydrate interaction can be applied to develop two types of nanoparticles: one incorporates lectins into nanoparticles as targeting moieties that are directed to cell-surface carbohydrates (direct lectin targeting), and the other is a reverse scenario in which carbohydrate moieties are coupled to nanoparticles to target lectins (reverse lectin targeting; ref. 57). An example of a drug conjugate using this particular reverse-lectin targeting is PK2, an actively targeted variant of the already developed PK1, in which the targeting moiety galactosamine is attached to the polymer backbone. Gamma-camera imaging showed that the PK2 targeting conjugate effectively targeted the liver whereas the nonconjugated counterpart (PK1) showed no targeting. PhaseI/II clinical trials have been completed in patients with primary or metastatic liver cancers (58).

## Potential of Nanoparticles to Overcome Drug Resistance

Drug resistance has emerged as a major obstacle limiting the therapeutic efficacy of chemotherapeutic agents. Among several mechanisms of drug resistance, P-glycoprotein is the best known and most extensively investigated (59). It has been suggested that nanoparticles may be able to circumvent P-glycoprotein–mediated resistance. One possible mechanism is that nanoparticles may avoid recognition by the P-glycoprotein efflux pump by means of being enveloped in an endosome when entering the cell, leading to high intracellular drug concentrations (Fig. 3; ref. 60). Ligand-targeted strategies, especially those using receptor-targeting ligands, may have particular potential for overcoming drug resistance because these ligands are usually internalized via receptor-mediated endocytosis. Indeed, a folate receptor–targeted, pH-sensitive polymeric micelle containing doxorubicin (61) and transferrin-conjugated paclitaxel-loaded nanoparticles (54) exhibited greater inhibitory activity against drug-resistant MCF-7 cells and/or xenografts than their nontargeted free drug counterparts.

## Future Direction and Opportunities

Together with the progression of nanoscale drug delivery systems, advances in nanoscale imaging suggest the potential for the development of multifunctional "smart" nanoparticles that may facilitate the realization of individualized cancer therapy. Almost all types of nanoparticles including polymeric nanoparticles (62), nanocrystals (63), polymeric micelles (19), dendrimers (64), and carbon nanotubes (65) have been evaluated for their suitability as multifunctional nanoparticles that can be applied for simultaneous in vivo imaging and treatment of cancers. Eventually, multiplex nanoparticles may be capable of detecting malignant cells (active targeting moiety), visualizing their location in the body (real-time *in vivo* imaging), killing the cancer cells with minimal side effects by sparing normal cells (active targeting and controlled drug release or photothermal ablation), and monitoring treatment effects in real time (Fig. 4).

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