Targeted for drug delivery

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The concept of targeted drugs is not new, but dates back to 1906 when Ehrlich[1] first postulated the 'magic bullet'. The durability of this concept is a strong indication of its appeal, but the 'magic bullet' continues to be a challenge to implement in the clinic. The challenge has been on three fronts: finding the proper target for a particular disease state; finding a drug that effectively treats this disease; and finding a means of carrying the drug in a stable form to specific sites while avoiding the immunogenic and nonspecific interactions that efficiently clear foreign material from the body. Nanoparticles are potentially useful as carriers of active drugs and, when coupled with targeting ligands, may fulfill many attributes of a 'magic bullet'. This review focuses on targeted drug delivery using nanoparticles as a modality that couples a ligand to a nanosized, drug-loaded vehicle as a potential means to achieve increased efficacy of a drug at the site of interest.

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Nanoparticulates encompass a variety of submicron (< 1 μm) colloidal nanosystems, which may be inorganic, liposome-based, or polymer-based (see the review by Gloria J. Kim and Shuming Nie in this issue)[2] – see Table 1. Nanoparticulate drug delivery systems have been studied for several decades now, and many of the features that make them attractive drug carriers are well known. One of the major advantages of nanoparticles is their small size, which allows them to pass through certain biological barriers.

A second advantage is that a high density of therapeutic agent can often be encapsulated, dispersed, or dissolved within these nanoparticles, which – depending on the preparation process – can be engineered to yield different properties and release characteristics for the entrapped agent. Because of the versatility of chemistries and preparation methods in these systems, surface functionalities can sometimes be incorporated into the nanoparticle. This facilitates additional attractive properties, such as the attachment of 'shielding' ligands that prolong the circulation of the nanoparticles in the blood stream, or the targeting of ligands for interaction with specific cells or tissue.

In this article we discuss two of the most widely used nanoparticulate systems in drug delivery: liposomes and solid biodegradable nanoparticles (Fig. 1).

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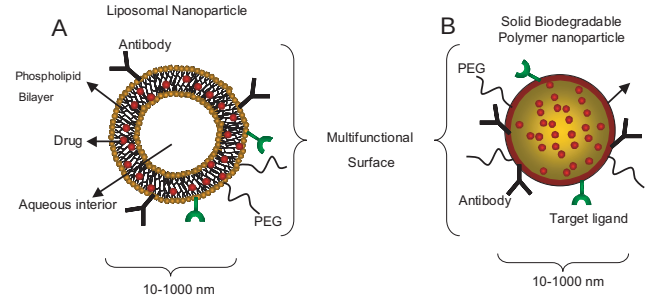
Liposomes

Liposomes as drug delivery vehicles were first proposed by Gregoriadis[3-6] and are a composition of amphiphilic phospholipids and cholesterol that self-associate into bilayers encapsulating an aqueous interior. These may be formulated into small structures (80-100 nm in size) that encapsulate either hydrophilic drugs in the aqueous interior or hydrophobic drugs within the bilayer (Fig. 1). Encapsulation is achieved using a variety of loading methods, most notably the pH gradient method used for loading vincristine[7] or the ammonium sulfate method for loading doxorubicin[8]. Additionally, the liposome surface can be engineered to improve its properties[9,10]. So far, the most noteworthy surface modification is the incorporation of polyethylene glycol (PEG) which serves as a barrier, preventing interactions with plasma proteins and thus retarding recognition by the reticuloendothelial system (RES)[11] and enhancing the liposome circulation lifetime. However, despite this versatility, there have been major drawbacks to the use of liposomes for targeted drug delivery, most notably, poor control over release of the drug from the liposome (i.e. the potential for leakage of the drug into the blood), coupled with low encapsulation efficiency, manufacturability at the industrial scale and poor stability during storage[12,13].



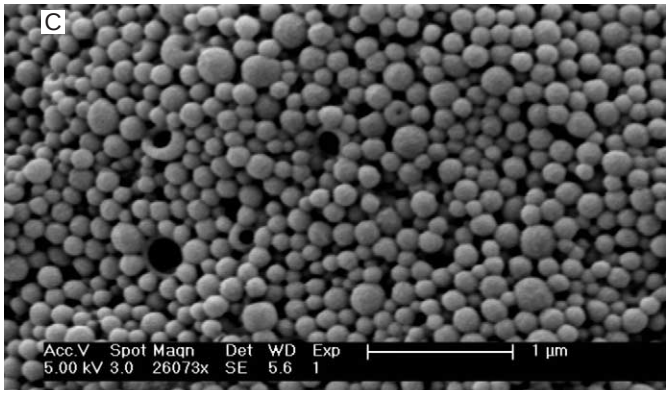


Fig. 1 Schematic of two different targeted nanoparticle drug delivery systems. (A) Liposomal-based systems are vesicular with targeting or PEG groups either preconjugated with a lipid then formed into a vesicle or postinserted into the liposome. Hydrophobic drug is encapsulated in the lipid bilayer. (B) Solid biodegradable nanoparticles are formed from a polymer emulsion with drug dispersed in a polymer matrix and targeting or PEG groups attached to an outer stablizing amphiphilic polymer shell. (C) Scanning electron micrograph of surface-modified PLGA nanoparticles.

Table 1 *Selected list of ligand-targeted nanoparticulate systems evaluated for in vitro or in vivo therapeutics delivery.*

Ligand	Drug	System	Target cells	Evaluation
Nucleic acids				
Aptamers[108]		PLA	Prostate epithelial cells	*In vitro*
ECM proteins				
Integrin[109]	Raf genes	Liposomes	Melanoma cells	*In vivo*
RGD peptides[110]	siRNA	Poly(ethylene imine)	Tumor vasculature	*In vivo*
Fibrinogen[111]	Radioisotopes	Albumin	Tumor vasculature	*In vivo*
Von Willebrand Factor[75,76] (Rexin-G®)	Cyclin gene	Viral particles	Pancreatic cancers	*In vivo*
Lipids				
MP Lipid A[97]		PLGA	Dendritic cells	*In vitro*
Carbohydrates				
Galactose[112]	Retinoic acid	PLA	Hepatocytes	*In vitro*
Hyaluronic acid[113]	Doxorubicin	Liposomes	CD44+ melanoma cells	*In vitro*
Peptidomimetics[114]	Various	mPEG/PLGA	Brain cells	Various
Antibodies to:				
HER2 receptor[115]		Gelatin/HAS	HER2 cells	*In vitro*
HER2 receptor[26]	Doxorubicin	Liposomes	HER2 cells	*In vivo*
CD19[29]	Doxorubicin	Liposomes	B cell lymphoma	*In vivo*
Vitamins				
Folate[116]	Doxorubicin	Liposomes	Leukemia cells	*In vivo*
Other				
Albumin[53,73,74] (Abraxane®)	Paclitaxel	Albumin-drug conjuate	Breast cancers	*In vivo*

Solid, biodegradable nanoparticles

Solid, biodegradable nanoparticles offer distinct advantages over liposomes. First, by varying the polymer composition of the particle and morphology, one can effectively tune in a variety of controlled release characteristics, allowing moderate constant doses over prolonged periods of time[14]. There has been a variety of materials used to engineer solid nanoparticles both with and without surface functionality[15]. Perhaps the most widely used are the aliphatic polyesters, specifically the hydrophobic poly (lactic acid) (PLA), the more hydrophilic poly (glycolic acid) PGA, and their copolymers, poly (lactide-co-glycolide) (PLGA). The degradation rate of these polymers, and often the corresponding drug release rate, can vary from days (PGA) to months (PLA) and is easily manipulated by varying the ratio of PLA to PGA. Second, the physiologic compatibility of PLGA and its hompolymers PGA and PLA have been established for safe use in humans; these materials have a history of over 30 years in various human clinical applications, including drug delivery systems[16,17]. Finally, PLGA nanoparticles can be formulated in a variety of ways that improve drug pharmacokinetics and biodistribution to target tissue by either passive or active targeting.

Ligand coupled nanoparticle features

Size and cellular uptake

The submicron size of nanoparticulates offers distinct advantages over larger systems. First, the small size enables them to extravasate through blood vessels and tissue. This is especially important for tumor vessels, which are often dilated and fenestrated with an average pore size of less than a micron compared with normal tissue[15,18-20]. Second, solid nanoparticles made from biodegradable polymers and encapsulated drug are ideal for sustained intracellular drug delivery, especially for drugs whose targets are cytoplasmic. An example of this application with dexamethasone-loaded nanoparticles locally delivered to vascular smooth muscle cells showed greater and sustained anti-proliferative activity compared with free drug, indicating more efficient interaction of the drug with cytoplasmic glucorticoid receptors[21,22].

Cellular internalization of drug-containing particles is likely to play a key role in determining their biological activity (Fig. 2). The molecular mechanisms mediating the internalization of particles are dependent on the size of the particles. Particles as large as 500 nm can be internalized by nonphagocytic cells via an energy-dependent process, which is inhibited by drugs that affect membrane vesicle formation. Smaller particles, with a diameter of less than 200 nm, are internalized via clathrin-coated pits, while larger particles are internalized via caveole membrane invaginations[23,24]. There might be other mechanisms mediating the internalization of particles that are independent of both clathrin and caveolae. Work on pathways that mediate the internalization of molecules and particles is an active area of research[25].

To facilitate efficient internalization, nanoparticles have been targeted against internalizing receptors and these have demonstrated increased therapeutic activity in some tumor models[26-30]. An example of this efficient internalization in

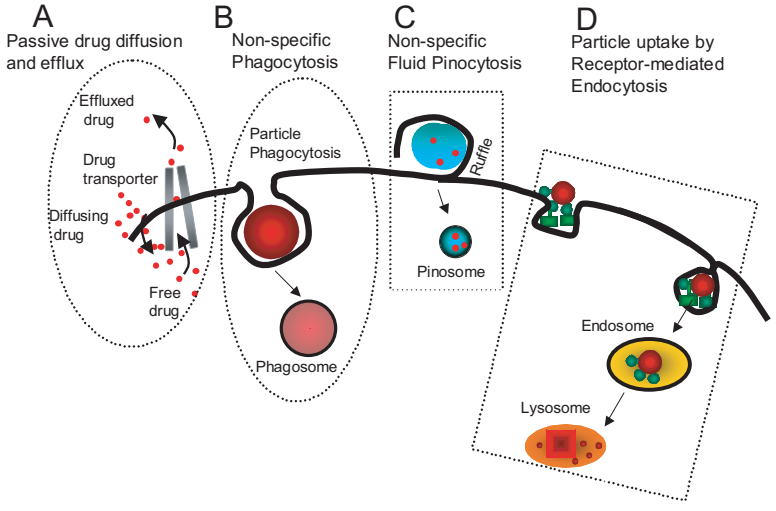


Fig. 2 Processes leading to cellular delivery of drug. A. Passive diffusion of free drug. B. Nonspecific phagocytosis of a nanoparticulate. C. Drug entrapped in fluid and uptake by pinocytosis. D. Receptor-mediated endocytosis. Nanoparticles bypass multidrug-resistant transporters that may efflux drug entering freely through the plasma membrane.

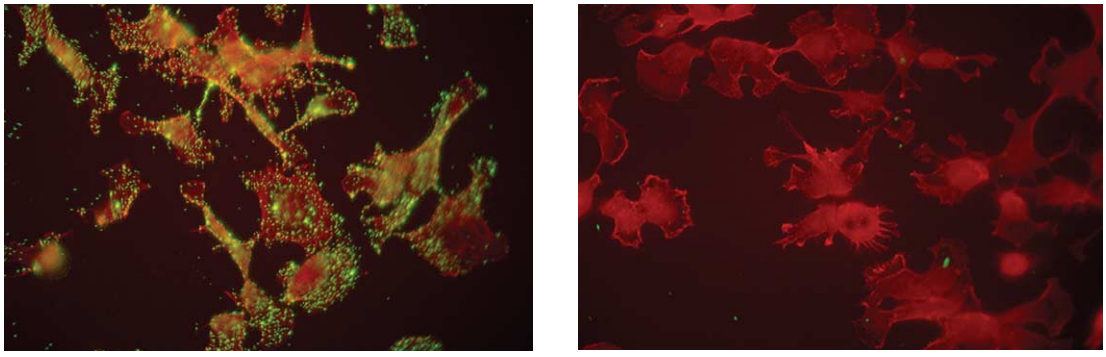


Fig. 3 An example of targeted nanoparticle internalization in tumor cells: 9L glioma brain tumor cells stained with 4 μg/ml anti-CD44 FITC-polystyrene nanoparticles (200 nm) (green) at 37°C (left) and counterstained with phalloidin-Texas red to reveal the cell shape (right).

tumor cells with anti-CD44 coated polystyrene nanoparticles is shown in Fig. 3. Liposomal nanoparticles loaded with doxorubicin, and targeted against the internalizing CD19 surface antigen on a B-cell lymphoma line, showed significant improvement over nanoparticles coupled to the noninternalizing anti-CD20 towards the same B-cell lymphoma cell line[31]. Similar results were achieved with folate-targeted cyanoacrylate nanoparticles, which internalized more efficiently in folic receptor bearing tumor cells versus cells devoid of the receptor[32].

Bypassing multidrug resistance

Nanoparticles also appear to be a useful approach for overcoming certain kinds of drug resistance. Some tumor cells are able to expel intracellular drugs into the external medium, thereby attaining resistance from drug action. This mechanism, called multidrug resistance, is related to the overexpression of the adenosine triphosphate (ATP)-binding cassette family of transporters, which include P-glycoprotein (Pgp) transporter and the multidrug resistance protein (MDRP) family[33,34]. These transporters are transmembrane proteins capable of pumping out many anticancer drugs that diffuse into the plasma membrane.

Because these transporters recognize drug in the plasma membrane, internalized particles bypass this mechanism and are able to release drug within the cytoplasm or endosomal vesicles, thereby increasing the effectiveness of the drug[35]. For example, it was shown that Pgp efflux affected the uptake of free doxorubicin compared to the uptake of folate-targeted liposomal doxorubicin in an MDR cell line[36]. In another study, it was also demonstrated that doxroubicin-cyanoacrylate nanoparticles were always more cytotoxic than free drug in rat glioblastoma cells[37]. Similar results demonstrate the enhanced ability of liposomal formulations of digoxin and vincristine to bypass the MDR pathways in a variety of cell lines[35,38]. Thus, it appears that the packaging of drug and delivery into the cell by way of endocytosis of nanoscopic materials may circumvent Pgp-mediated MDR, abrogating the need for coadministration of Pgp inhibitory agents (see Fig. 2).

Enhanced interactions with target through multivalency

An important feature of targeted particle delivery is the ability to simultaneously carry a high density of drug while displaying ligands on the surface of the particle. It is well known that other drug carrier systems, such as immunotoxins or drug-immunoconjugates, which are made by tethering drug molecules to antibodies or synthetic polymers, usually deliver less than ten drug molecules per carrier to target cells. Targeted nanoparticles, on the other hand, can deliver thousands of drug molecules.

In addition, the overall strength of nanoparticles binding to a target is a function of both the affinity of the ligand-target interaction and the number of targeting ligands present on the particle surface. This is a particularly useful feature for ligands that, in their monomer form, have a weak affinity to their target receptors, such as single-chain variable fragments (scVF). In most cases, these must be reengineered into multimers to increase their avidity of interaction to target cells[39-41] or peptide/Major histocompatability complex (peptide/MHC), which have weak affinity to target T cell receptors. In our work, for example, we found that this multivalency increased the avidity of interaction of peptide/MHC to the T cell up to 100-fold facilitating enhanced interactions and effective drug delivery to target antigen-specific T cells[42].

Synergistic effects between target ligand and encapsulated drug

Certain monoclonal antibodies, when combined with a drug, can prove to be more beneficial than the drug alone or

antibody alone in inhibiting the proliferation of cancer cells. Synergistic or additive therapeutic effects can occur with the simultaneous presentation of antibody and drug to target cells; this can be translated into a single unit when nanoparticles encapsulating drug are also surface modified to present a synergistic ligand. A majority of the target ligands are monoclonal antibodies, which can bind to their target and also initiate signaling cascades that may lead to apoptosis[43], blockade of multidrug resistance[44], induction of cell cycle arrest[45], and inhibition of DNA repair in target cells[46]. When combined with chemotherapeutic drugs, the therapeutic efficacy of established monoclonal antibodies is enhanced. As an example, the therapeutic efficacy of Rituxan, anti-CD20, is enhanced when coadministered with chemotherapeutic drugs[47]. Other studies have demonstrated the synergistic effect of chemotherapy with the HER-2/neu targeted antibody against breast cancers, trastuzumab[48,49], and the synergy between chemotherapy and antibody treatments in the treatment of other established solid tumors[50].

While these studies support the beneficial role of additive effects in treating disease by coadminstration of antibodies and chemotherapeutic drugs, there are no studies so far that show that these effects could be achieved with nanoparticles encapsulating drug and displaying target ligands. Synergistic effects that may be exploited by targeted nanoparticulates thus represent a potential that has not yet been tapped.

Methods for coupling targeting ligands to nanoparticles

Targeting ligands include any molecule that recognizes and binds to target antigen or receptors overexpressed or selectively expressed by particular cells or tissue components. These may include antibodies or their fragments, peptides, glycoproteins, carbohydrates, or synthetic polymers (Table 2). The most widely used coupling group is PEG, because this group creates a hydrophilic surface that facilitates long circulation of the nanoparticles. This strategy has been used successfully in making 'stealth' liposomes with affinity towards target cells[51]. The incorporation of ligands into liposomes is easily achieved by conjugation to the phospholipid head group – in most cases this is phosphotidylethanolamine (PE). The strategy relies either on preinsertion of the functionalized lipid or postinsertion into a formed liposome[9,52]. Functionality could also be introduced by incorporating PEG with functional endgroups for coupling to target ligands.

Table 2 *Materials used to prepare biodegradable and vesicular nanoparticulate systems.*

Type of system	Material/Name
Solid biodegradable polymers	Gelatin[115,117,118]
	Chitosan[119]
	Albumin[120,121]
	Polyesters PLA, PGA, PLGA[22]
	PEG-polyester block copolymers[122,123]
	Poly(alkylcyanoacrylates)[124]
	Polyanhydrides[125]
	Polycaprolactone[126-129]
	Solid lipid[130,131]
Vesicular systems	Liposomes[132]
	Niosomes[133]
	Polymer micelles and polymersomes[134,135]

The situation is slightly more complicated with solid nanoparticles. One strategy involves the formulation of target-drug conjugates into nanoparticles; an example is the US Food and Drug Administration (FDA)-approved albumin-paclitaxel nanoparticles system (Abraxane®) used for treatment of advanced breast cancer[53]. In the case of PLGA, a major difficulty has been the lack of functional chemical groups on the aliphatic polyester backbone for linking to target ligands. To introduce functionality into PLGA surfaces, several approaches have been studied. These include synthesis of PLGA copolymers with amine[54,55] or acid[55] end-groups; these new polymers are subsequently fabricated into particles. Another approach involves the blending or adsorption of functional polymers such as polylysine[56,57], poly(ethylene-alt-maleic acid) (PEMA)[58], or PEG[59] into PLGA and forming particles and matrices from these blends[57,58,60-62]. Plasma treatment of the PLGA matrix has also been proposed for the purpose of modifying its surface properties and introducing hydrophilic functional groups into the polymer[63,64]. These techniques have suffered from their complexity as well as low densities of targeting molecules achieved. As an alternate strategy, others have linked tumor targeting ligands such as folic acid to distearylethanolamine (DSPE) via a PEG spacer and incorporated this into lipid nanoparticles[65]. Our group has introduced functional fatty acids that can be incorporated into the particle during the formation process. This strategy facilitated a high density of incorporated ligands and prolonged presentation, while maintaining sustained delivery of encapsulated agent at the target site[66].

Targeting modalities

In vivo passive targeting

One of the most promising applications for targeted drug delivery using nanoparticles is in local application using interventional procedures such as catheters. Potential applications have focused on intra-arterial drug delivery to localize therapeutic agents in the arterial wall to inhibit restenosis[67,68]. Restenosis is the re-obstruction of an artery following interventional procedures such as balloon angioplasty or stenting. Drug-loaded nanoparticles are delivered to the arterial lumen via catheters and retained by virtue of their size, or they may be actively targeted to the arterial wall by nonspecific interactions such as charged particles or particles that target the extracellular matrix. Indeed, nanoparticles loaded with dexamethasone and passively retained in arteries showed reduction in neointimal formation after vascular injury[69].

Passive delivery may also be targeted to tumors. Aggressive tumors inherently develop leaky vasculature with 100-800 nm pores due to rapid formation of vessels that must serve the fast-growing tumor. This defect in vasculature coupled with poor lymphatic drainage serves to enhance the permeation and retention of nanoparticles within the tumor region. This is often called the EPR effect[70,71]. This phenomenon is a form of 'passive targeting'. The basis for increased tumor specificity is the differential accumulation of drug-loaded nanoparticles in tumor tissue versus normal cells, which results from particle size rather than binding. Normal tissues contain capillaries with tight junctions that are less permeable to nanosized particles. Passive targeting can therefore result in increases in drug concentrations in solid tumors of several-fold relative to those obtained with free drugs[2]. While this effect has been observed, it is unclear if it can be exploited for targeting of nanoparticle drug formulations. Surface-modified nanoparticles, engineered to display an overall positive charge facilitated adhesion to the negatively charged arterial wall and showed a seven- to ten-fold greater arterial localized drug levels compared with the unmodified nanoparticles in different models. This was demonstrated to have efficacy in preventing restenosis of coronary arteries (i.e. constriction after corrective surgery) in dogs and pigs[67]. This application highlights the promising potential for improved drug delivery with drug-loaded nanoparticles to local sites using common catheterization procedures for the prevention of pathologies connected to interventional procedures such as angioplasty.

Passive delivery may also be directed to lymphoid organs of the mammalian immune system, such as lymphatic vessels and spleen. These organs are finely structured and specialized in eliminating invaders that have gained entry to tissue fluids. Nanoparticles may easily penetrate into lymphatic vessels, taking advantage of the thin walls and fenestrated architecture of lymphatic microvessels. Passive targeting to the spleen is via a process of filtration. Indeed, the spleen filters the blood of foreign particles larger than 200 nm. This function facilitates splenic targeting with nanoparticles encapsulating drug for effective treatments against several hematological diseases[72].

Both liposomal and solid nanoparticles formulations have received clinical approval for delivery of some anticancer drugs. Examples of liposomal formulations include doxorubicin (Doxil1/Caelyx1 and Myocet1) and daunorubicin (Daunosome1). The mechanism of drug release from liposomes is not clear, but is thought to depend on diffusion of the drug from the carrier into the tumor interstitium. This is followed by the subsequent uptake of the released drug by tumor cells. The mechanism of release is still poorly understood, which hinders advanced applications involving the addition of active ligands for cellular targeting *in vivo*.

Recently, the FDA-approved Abraxane®, an albumin-bound paclitaxel nanoparticle formulation in an injectable suspension for the treatment of metastatic breast cancer[53,73,74]. In addition, other solid nanoparticle-based cancer therapies have been approved for clinical trials. For example, a phase 1 clinical trial has been approved that will evaluate the safety of a hepatic arterial infusion of Rexin-G® (a targeted nanoparticle vector system with a proprietary mutant cell-cycle control gene, i.e. anti-cancer gene) as an intervention for stage IV metastatic pancreatic cancers[75,76].

Targeting by route of adminstration

The selection of the route of adminstration for nanoparticles can be critical for successful targeting. One important distinction is the direct administration to a physically local region of tissue versus indirect delivery via the systemic circulation. For example, the oral administration of particles is an attractive approach for direct targeting of intestinal mucosal sites, such as gut-associated lymphoid tissue (GALT) for the delivery of protein antigens for vaccination. At the same time, oral administration has the potential for sustained noninvasive drug delivery via the systemic circulation. Sustained delivery by the oral route, however, is challenging

because of the stomach's corrosive environment and the barriers that restrict prolonged absorption into the circulation. To bypass these barriers, oral doses incorporate enteric coatings, which allow particles to pass the stomach unharmed, include permeation enhancers[77,78], or add ligands that target the intestinal epithelium or the lymphoid Peyers patches[79,80]. Greater bioavailability can sometimes be engineered into nanoparticles by milling a high density of drug into nanocrystals, which dissolve rapidly in the stomach[81]. For example, Rapammune® is made from rapamycin and is being used successfully for preventing organ rejection after transplants.

Other important routes of delivery include transdermal delivery (for direct administration to skin and hair follicles in applications ranging from alopecia[82] to genetic immunization[83,84]) and pulmonary delivery of aerosolized nanoparticles (for the prevention of asthma[85] or the rejection of lung transplants). A notable example here is aerosolized cyclosporine, which has been shown to have a greater therapeutic efficacy in preventing acute lung transplant rejection when delivered by inhalation[86]. Ocular delivery of drug-loaded, sustained-release nanoparticles by intravitreal adminstration is a promising route for eye disease, because it eliminates the need for multiple injections of drug into the eye. Coupled with the problem of retention of adequate concentrations of therapeutic agent in the eye[87], biodegradable nanoparticles delivered intravitreally have demonstrated localization in the retinal pigment epithelium[88] and greater therapeutic efficacy in ocular disease such as autoimmune uveoretinitis[89]. An ophthalmic formuluation of Rexin-G®[76] (Hazin-G®, a targeted viral vector bearing inhibitory genes) is currently under consideration by the FDA, for use in a proposed phase I clinical trial for the reduction of severe cases of corneal scarring.

In vivo active targeting

Target ligands attached to the surface of nanoparticles may act as 'homing devices', improving the selective delivery of drug to specific tissue and cells. This is especially true for targets that are readily accessible from the vasculature, e.g. circulating malignant cells in hematological malignancies such as B cell lymphoma and multiple myeloma[28,29]. When tumor cells were administered intravenously in mice, active targeting of nanoparticles has been noted to increase the therapeutic index of drug when the tumors were just beginning to be established[90,91]. Liposomal doxorubicin (Doxil®), which incorporates an antibody that targets a growth factor overexpressed by breast tumors (ErbB2), showed a faster and greater regression in tumor volume compared to unmodified Doxil[92].

One important target has been E-selectin, which is involved in the arrest of circulating immune system cells and is differentially upregulated with inflammatory and immune processes[93,94]. Indeed, this molecule has become a potential target for several strategies designed to enhance delivery of therapeutic agents to the vasculature, including tumor blood vessels through selective targeting. A second important class of targets is receptors involved in the uptake of vitamin B12, folic acid, biotin, and thiamine[95]. These are differentially overexpressed on the surface of cancer cells, which creates a possible target for several types of cancer, including ovarian, breast, lung, renal, and colorectal cancers[96].

One of the most promising strategies for enhancing active immunotherapy and inducing potent vaccination is the targeting of antigen-loaded nanoparticles to antigen-presenting cells such as dendritic cells (DCs)[97]. Toward that goal, nanoparticles incorporating toll-like receptors (TLRs) in biodegradable PLGA have shown efficient delivery of antigen to DC and potent activation of the T cell immune response[98].

Many other potential targets for therapy have been proposed, and some believe that targeted nanoparticles could emerge as an important 'generic' strategy for delivering agents to specific sites in the body. Still, much work remains to be done to understand the pharmacokinetics of targeted nanoparticle delivery and its application in clinical settings.

Barriers to tumor targeting *in vivo*

Targeting nanoparticles to tumor cells *in vivo* has proven challenging for several reasons. First, within a solid tumor, targeted nanoparticles encounter a phenomenon known as the 'binding site barrier'[99], in which particles tend to bind to cells at the periphery of the tumor mass, slowing further diffusion within the tumor. A second factor relates to heterogeneity of the tumor and its vasculature. Vascularization of tumors is heterogonous, with some cells not expressing the same epitopes as those being targeted, regions that are necrotic and other regions that are well vascularized[100], an interstitium that is characterized by high interstitial pressure, resulting in an outward convective fluid pressure[101], and the absence of regular lymphatic vessels[102]. Shedding of the target receptors and downregulation further

complicates matters. Thus, the resistance of tumors to therapeutic intervention is a result of both physiochemical alterations (in the form of abnormal composition and structure) and biochemical alteration (in terms of the abnormal expression of cell surface receptors).

As a result, it has been observed that active targeting may confer no additional benefits to the therapeutic index of drug if solid tumors are established. One group observed that targeted and nontargeted liposomal doxorubicin gave similar results when treating established solid tumors, although the results were better than treatment with free drug[90,103]. Another group that showed similar indiscriminate results with anti-HER2 targeted liposomes and nontargeted liposomes[26].

Future directions

The current focus in pharmaceuticals is shifting to a 'smart drug' paradigm, in which increased efficacy and decreased toxicity are the motivating factors. We envision that this could be achieved with targeted nanoparticles, where repertoires of targets and a series of drugs could yield new generations of highly specific therapeutic agents. In many respects this vision is well underway, with over 200 clinical trials for various antibody-containing formulations[104].

The most promising application of *in vivo* targeted nanoparticle drug delivery currently involves readily accessible targets in the vasculature, such as malignant immune system cells. A second promising application is in local attachment via interventional catheterization procedures to the vasculature or other tissues. For *in vivo* targeting of other sites, the characteristics of the targeted organ or cells are important. For example, targeted delivery to the lung is readily achievable, because lung capillary beds are the first to be encountered following an intravenous injection of the particles, and particles are often physically retained in the vascular bed. Additional sites also appear practical: the liver is a massive clearing house for most particles in circulation, and the spleen is a blood filter. Delivery to the lymph nodes could easily be achieved with intradermal or subcutaneous injection that avoid circulation. In cases of bacterial or viral invasions, where cells of the RES are compromised, the clearance of nanoparticles by those cells is actually of therapeutic importance. Thus the route of adminstration is of critical importance to the success of targeting.

However, in cases where remote organs or cells (such as cells within solid tumors) are the targets, there is still a need to find ways to navigate nanoparticles through the labyrinth to the target site while avoiding clearance. Toward that goal, there have been reports of using hydrophilic coatings, such as PEG, polaxamers, and polyamines, to achieve enhanced circulation time[105-107]. Even with breakthroughs in the engineering of long-circulating nanoparticles, there is still the additional challenge of understanding and achieving the dosing that delivers consistent pharmacokinetics. There is no doubt that, with additional understanding of pharmacokinetics and immunity combined with the development of novel biomimetic strategies[79], these hurdles will be translated into practical therapeutics in the clinic. NT

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