Receptor-targeted nanocarriers for therapeutic delivery to cancer

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Abstract

Efficient and site-specific delivery of therapeutic drugs is a critical challenge in clinical treatment of cancer. Nano-sized carriers such as liposomes, micelles, and polymeric nanoparticles have been investigated for improving bioavailability and pharmacokinetic properties of therapeutics via various mechanisms, for example, the enhanced permeability and retention (EPR) effect. Further improvement can potentially be achieved by conjugation of targeting ligands onto nanocarriers to achieve selective delivery to the tumour cell or the tumour vasculature. Indeed, receptor-targeted nanocarrier delivery has been shown to improve therapeutic responses both \textit{in vitro} and \textit{in vivo}. A variety of ligands have been investigated including folate, transferrin, antibodies, peptides and aptamers. Multiple functionalities can be incorporated into the design of nanoparticles, e.g., to enable imaging and triggered intracellular drug release. In this review, we mainly focus on recent advances on the development of targeted nanocarriers and will introduce novel concepts such as multi-targeting and multi-functional nanoparticles.

Keywords

Drug delivery; nanocarriers; drug targeting

Introduction

Cancer is a leading cause of death in the US and around the world. A majority of anticancer agents in clinical use today are chemotherapeutics given systemically. These are toxic not only to cancerous cells but also to proliferating normal cells, such as those of the bone marrow and the intestinal epithelium. This can lead to severe side-effects and treatment failure. Therefore, improving the therapeutic index by increasing therapeutic effects to tumour cells and decreasing toxicity to healthy tissues is a central issue in improving cancer therapy. One strategy towards this goal is to develop new drugs that specifically interfere
with intracellular pathways exclusive to cancer cells (Neidle and Thurston 2005). For example, a tyrosine kinase inhibitor imatinib (Gleevec) targeting the BCR-ABL oncogene was successfully used in treating chronic myelogenous leukaemia (CML) (Allen 2002, Sawyers 2004). In addition, a number of therapeutic monoclonal antibodies have entered clinical use, e.g., trastuzumab (Herceptin) targeting HER2 receptor overexpressed in a subset of breast cancer (Allen 2002, Sawyers 2004). An alternative strategy for developing a targeted drug is to design a receptor-targeted delivery system, which either can be based on direct coupling of an anti-cancer drug to a ligand or based on encapsulation of the drug into a ligand-directed drug carrier (Arap et al. 1998, Allen 2002, Torchilin 2007). For example, Mylotarg, an anti-CD33-calicheamicin immunoconjugate, has been developed for the treatment of acute myelogenous leukemia (AML) (Sharkey and Goldenberg 2006, 2008). In general, however, immunoconjugates have a relatively low achievable drug/antibody ratio (usually 3 ~ 10 drug molecules/antibody). Only a few extremely potent drugs can be targeted in this fashion, precluding the use of this delivery technology on most existing chemotherapeutic agents (Allen 2002, Sharkey and Goldenberg 2008). Furthermore, direct conjugation of drugs to the targeting ligand may adversely impact the targeting molecule by disrupting ligand/receptor recognition and potentially alter the cytotoxicity of the drug (Allen 2002, Noble et al. 2004). As an alternative, receptor-targeted nanocarrier drug delivery system is an emerging platform in development of cancer therapy (Arap et al. 1998, Allen 2002, Sapra and Allen 2003, Noble et al. 2004, El Bayoumi and Torchilin 2009).

Nano-sized carriers (10 ~ 400 nm) are desirable as drug carriers because they possess the advantages of being capable of carrying large amount of drugs, having prolonged circulation time (especially when surface PEGylated), and facilitating selective tumour accumulation via the enhanced permeability and retention (EPR) effect (Jain 1999, Allen and Cullis 2004, Alexis et al. 2008, Soussan et al. 2009). Nanocarriers are also helpful in addressing other limitations of conventional drugs, including poor aqueous solubility, low bioavailability and/or unfavourable pharmacokinetic properties. In addition, delivery via nanocarriers has been reported to overcome multidrug resistance (MDR) caused by drug efflux transporters such as the P-glycoprotein, which are frequently overexpressed in cancer cells (Gottesman et al. 2002, Piddock 2006). To date, a variety of nanocarriers (e.g., liposomes, micelles, and polymeric nanoparticles) have demonstrated efficacy both in vitro and in vivo (Allen and Cullis 2004, Duncan 2006, Peer et al. 2007, Davis et al. 2008, Jagur-Grodzinski 2009). To achieve higher specificity, nanocarriers can be surface modified with ligands that specifically recognize receptors on tumour cells. Combining passive and active targeting in a single platform may further improve the therapeutic index of nanocarrier delivered drugs.


**Design of targeted nanocarriers**

The feasibility of selective and efficient delivery of anticancer therapeutics using nanocarriers has been demonstrated in numerous studies. There are two major mechanisms: Passive targeting and active targeting. Important factors to be considered include carrier composition and selection of targeting ligand.
Passive versus active targeting

In contrast to normal tissues, many solid tumours possess unique structural features of hyperpermeable vasculature and impaired lymphatic drainage (Matsumura and Maeda 1986, Hobbs et al. 1998). As a result, tumour tissues are relatively permeable to macromolecules and nanocarriers (Jain 1999, Torchilin 2005b, Gu et al. 2007, Peer et al. 2007, Gullotti and Yeo 2009, Shi et al. 2009b). Passive targeting, therefore, refers to the selective extravasation and retention of long-circulating nanocarriers at tumour sites due to the enhanced permeability and retention (EPR) effect. In contrast, active targeting is based on specific interactions between the nanocarrier and receptors on the target cell, which may also promote internalization of nanocarriers through receptor mediated endocytosis (Figure 1).

To take full advantage of the EPR effect, it is critical to incorporate several properties into the design of nanocarriers. A key consideration is the need for long circulation time in the blood stream, required for extravasation. It has been shown that the threshold size for extravasation in tumours is ~400 nm in diameter, and that nanocarriers with diameters of less than 200 nm are preferred (Yuan et al. 1995, Hobbs et al. 1998). On the other hand, it is known that the kidneys are capable of filtering particles significantly smaller than 10 nm (about 70,000 Da) (Caliceti and Veronese 2003, Alexis et al. 2008). Therefore, the particle size of nanocarrier should be between 10 and 200 nm for tumour delivery. Surface charge of nanocarriers is another important parameter. Both highly positive and highly negative charged nanocarriers are susceptible to rapid clearance by the reticuloendothelial system (RES) (Li and Huang 2008). Thus, it is important to design nanocarriers with either a neutral or a slight negative zeta potential. In addition, a common method for reducing the recognition of nanocarriers by the RES is to coat their surfaces with polyethylene glycol (PEG) (Caliceti and Veronese 2003, Alexis et al. 2008). Due to steric effect of the hydrophilic PEG, the binding of nanocarriers to opsonins, which promotes RES clearance, is significantly reduced, resulting in prolonged circulation time and increased accumulation at the tumour sites via EPR.

Passive targeting only facilitates the efficient localization of nanocarriers in the tumour interstitium. It cannot further promote their uptake by cancer cells. For this reason, receptor-based active targeting strategies are being investigated for nanocarriers. In addition to specific interactions between the ligands on the surface of nanocarriers and receptors expressed on the tumour cells, this may trigger receptor-mediated endocytosis. Furthermore, active targeting has shown the potential to suppress multidrug resistance (MDR) via bypassing of P-glycoprotein-mediated drug efflux (Gottesman et al. 2002, Gu et al. 2007, Peer et al. 2007).

Interestingly, many studies have revealed no significant differences in the overall levels of tumour accumulation between non-targeted nanocarriers and receptor targeted nanocarriers (Alexis et al. 2008, Gullotti and Yeo 2009). This is because both types of nanocarriers can selectively reach tumour sites via EPR. The targeting ligands may not play a role until the targeted nanocarriers extravasate at the tumour sites. Nevertheless, the therapeutic efficacy of anticancer drugs in targeted nanocarriers can be improved dramatically due to receptor-mediated internalization and intracellular drug release. Notable exceptions to this include when the target is on the tumour endothelial cells rather than the tumour cells, and when the targeted cells reside in the vascular compartment or in tissues with high accessibility to the vasculature. In these situations, targeting occurs relatively quickly and does not require extravasation of the nanocarriers. As an example of vasculature targeting, there have been numerous reports of nano-carriers conjugated to cyclic RGD peptide (targeting neovasculature marker \( \alpha_v \beta_3 \) integrin), VEGF or anti-VEGFR (targeting VEGFR), or antibody or ligand targeting the prostate specific membrane antigen (PSMA). These have shown significant improvements in therapeutic efficacy \textit{in vivo}. Meanwhile, various types of
leukemia constitute examples of target cells having high accessibility from the circulation without the requirement for extravasation due to the vascular localization of the leukemia cells. In fact, most therapeutic antibodies approved to date have been targeting leukemias (e.g., rituxan, zevalin, bexxar, campath, mylotarg).

**Commonly used nanocarriers**

Nanocarriers refer to nano-sized particles that are capable of carrying drugs. Several classes of materials have been developed for nanocarriers, including lipids (liposomes), biocompatible polymers (e.g., polymeric nanoparticles), and surfactants (micelles) (Duncan 2003, Allen and Cullis 2004, Torchilin 2005b, Duncan 2006, Farokhzad and Langer 2009, Soussan et al. 2009) (Figure 2). Drugs can be encapsulated in a vesicle, entrapped in a matrix, or solubilized within a hydrophilic or a hydrophobic component (Bonacucina et al. 2009, Mishra et al. 2010).

Liposomes are self-assembling vesicles composed of lipid bilayers surrounding an aqueous compartment. Hydrophilic drugs are readily encapsulated in the aqueous core while lipophilic drugs are solubilized within the lipid bilayer (Torchilin 2005b, Abu Lila et al. 2009). Liposomes carrying chemotherapeutic drugs such as doxorubicin (Doxil®) and daunorubicin (DaunoXome®) have been approved by FDA since the mid-1990s (Lammers et al. 2008). Micelles are composed of surfactants such as amphiphilic block-copolymers that self-assemble into nano-aggregates (5–50 nm). Drugs can be entrapped in the core of micelles (Torchilin 2005a, Aliabadi and Lavasanifar 2006, Peer et al. 2007). SP1049C, a micelle formulation of doxorubicin comprising pluronics, and NK911, another micelle formulation of doxorubicin comprising polyethylene glycolopoly (aspartic acid) block copolymer have been studied in clinical trials (Danson et al. 2004, Matsumura et al. 2004). Dendrimers are well-defined branched macromolecules with high molecular uniformity and narrow polydispersity. Hydrophobic drugs can be entrapped in the hydrophobic interior of dendrimers to promote solubilization. Drugs can also be covalently conjugated onto the surface of the dendrimer (Duncan and Izzo 2005, Nanjwade et al. 2009). Polymeric nanoparticles are generally nano-sized polymeric matrix by which drug can be physically entrapped via the association between the drug and polymer or chemically conjugated through the covalent bond between the drug and polymer (Duncan 2006, Jagur-Grodzinski 2009). Alternative to polymeric nanocarriers, several polymeric macro-molecular conjugates, such as Oncaspar (PEG-L-asparaginase), PEG-INTRON (PEG-α-interferon 2b), and Zinostatin (Styrene maleic anhydride), have been clinically approved for the treatment of various types of cancers (Duncan 2003).

**Targeting ligands for nanocarriers**

Various types of targeting ligands have been exploited for constructing targeted nanocarriers (Table I), as discussed in recent reviews (Allen 2002, Sapra and Allen 2003, Noble et al. 2004, Gu et al. 2007, Phillips et al. 2008, Vives et al. 2008, Yan and Levy 2009, Veiseh et al. 2010). The targeted receptor can either be tumour-specific or tissue-specific. Tumour-specific ligands also include receptors upregulated in tumour-associated vasculature. It may not be very feasible to identify unique receptors expressed only on tumours but not on normal cells. Therefore, tissue-specific ligands or ligands overexpressed on tumour cells are frequently used, which are designed to reduce side-effects to other normal tissues. For examples, galactose derivatives, which targets the asialoglycoprotein receptor (ASGPR) (Wu et al. 2002) (expressed on hepatocytes and on hepatoma cells), rituximab, which targets CD20 (Allen 2002) (expressed on normal and malignant B cells), and transferrin (Tf), which targets the Tf receptor (Qian et al. 2002, Daniels et al. 2006) (expressed on normal tissues and, often at elevated levels on tumour cells) have proven to be useful ligands for tumour targeting. Other important factors to consider include expression level of receptor on
tumours, internalization capacity and rate, receptor binding affinity, ligand sizes, immunogenicity, as well as availability. In general, nanocarriers with internalizing antibody show better delivery efficiency than those with non-internalizing antibody (Allen 2002). However, in some cases, a non-internalizing antibody that only binds on cell surface may have the benefit of promoting greater bystander effects and enabling immunological mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC) (Clynes et al. 2000). Examples of potential targeting ligand are folate, transferrin, antibodies and peptides. Folate receptor (FR) is frequently overexpressed in a number of human tumours such as ovarian, colorectal and breast cancer, while not expressed in most normal tissues (Low et al. 2008, Zhao et al. 2008). Thus, FR is a highly selective tumour marker. Linking folic acid to liposomes or polymers has been shown to facilitate tumour-specific delivery of anticancer drugs. Similar to other low molecular weight ligands, folic acid has advantages of simple conjugation chemistry, non-immunogenicity and low cost. Transferrin receptor (TfR) is highly upregulated on many tumour cells due to their higher demand for iron (Daniels et al. 2006). TfR-mediated delivery can be obtained via the chemical conjugation of Tf or anti-TfR antibody or antibody fragments onto nanocarriers. Due to the high specificity and affinity of antibody-antigen interaction, Ab mediated delivery is an attractive means for designing targeted nanocarriers. Besides whole Abs, antibody fragments such as Fab and scFv can be used for constructing targeted nanocarriers (Allen 2002). Potential problems include immunogenicity, high cost and large ligand size. As an alternative to antibodies, peptides have gained much attention as targeting ligands because of their small size, lower immunogenicity and higher stability as well as easy manufacture (Vives et al. 2008). For instance, cyclic versions of the arginine-glycine-aspartic acid (RGD) peptide that targets to \( \alpha_v \beta_3 \) integrin receptor has been used as a targeting ligand to tumour vasculature (Sipkins et al. 1998, Ruoslahti et al. 2010). The \( \alpha_v \beta_3 \) integrin is highly expressed in tumour vasculature and various metastatic cancer cells (Ruoslahti 2002, Desgrozillier and Cheresh 2010). The use of new screening technologies such as phage display has led to the discovery of novel peptides with high binding affinity and specificity towards various cells, tissues and organs (Balestrieri and Napoli 2007, Vives et al. 2008).

**Conjugation strategies in targeted nanocarriers**

The construction of targeted nanocarriers involves the association of targeting ligands on the nanocarriers through either chemical conjugation or physical interaction. Chemical conjugation provides a stable linkage between targeting ligands and nanocarriers. The conjugation can be performed either before or after nanocarrier formation. Thioether, disulfide, and amide covalent linkages are frequently incorporated into the chemical conjugation of ligands on the surface of nanocarriers (Nobs et al. 2004). To maximize the targeting efficiency, PEG is usually used as a linker to increase the distance between nanocarriers and targeting ligands, thereby reducing the steric interference of nanocarriers to receptor binding. A streptavidin-biotin affinity-based non-covalent conjugation strategy also has frequently been used in targeted nanocarriers (Nobs et al. 2004, Shi et al. 2009b).

**Recent advances in targeted nanocarriers**

New tumour targets and tumour vasculature targets are being discovered and developed for nanocarrier targeting based on the use of novel technologies such as protein engineering, phage display and aptamer screening. A number of novel concepts have been investigated in developing targeted nanocarriers, e.g., a dual receptor targeting strategy that combines two ligands on the same nanocarriers (Laginha et al. 2005, Saul et al. 2006), and an organelle-targeted delivery strategy (Torchilin 2006b, Yousif et al. 2009). Moreover, novel designs have integrated more functions in nanocarriers, including simultaneous delivery of anticancer drugs and nucleic acids (e.g., siRNA), imaging agents (e.g., quantum dots), and stimuli-responsive components (e.g., light, temperature and pH-sensitive materials).
Torchilin 2006a, 2009, Ganta et al. 2008, McCarthy and Weissleder 2008, Farokhzad and Langer 2009, Patil et al. 2010, Veiseh et al. 2010). These multifunctional nanocarriers can be used to visualize their distribution in the body by non-invasive imaging methods, and can possibly monitor treatment response in real time. The combination of diagnostic and therapeutic functions in nanoparticles (theranostic nanoparticles) represents a new trend in academic research in the area.

**Development of novel ligands for targeted nanocarriers**

**Novel ligands for tumour and tumour vasculature targeting**

Representative novel ligands for targeted nanocarriers and their application in anticancer therapy are listed in Table II. Generally, receptors for endogenous ligands consist of transferrin receptor, carbohydrates (lectins) receptors, hormone receptors, mannos receptor, asialoglycoprotein (galactose) receptor, lipoprotein receptors, macroglobulin receptor, epidermal growth factor (EGF) receptor, platelet-derived growth factor (PDGF) receptor, vasculature receptors and insulin receptor. Antibodies are often used for construction of targeted nanocarriers. As illustrated in Table II, some novel Abs like anti-Tf, A7 antibody and 5D4 antibody et al. are used in targeted nanocarriers (Sharkey and Goldenberg 2008). Whole antibodies have poor tumour penetration and may promote clearance via FcR by macrophages (Carter 2001). Therefore, antibody fragments including antigen-binding fragments (Fab) and single-chain variable fragment (scFv) can be used. For example, a low-affinity scFv against the ErbB2 has been shown to enhance tumour localization of nanocarriers in a mouse tumour model (De Lorenzo et al. 2002). More recently, newer classes of engineered proteins or protein-like molecules have been developed as targeting ligands for targeted nanocarriers. These include affibody (a small protein domain) (Wikman et al. 2004), avimers (small protein acting as antibody) (Silverman et al. 2005) and nanobody (a heavy-chain evolved from antibody) (Cortez-Retamozo et al. 2004). In comparison with intact antibodies, these smaller molecules have been shown as enhanced tumour penetration and lower immunogenicity. Derived from the phage display technology, a 7 kD affibody molecule is selected to bind EGFR receptor with sub-nanomolar affinity (Wikman et al. 2004). Beuttler et al. (2009) reported that anti-EGFR affibody molecule can be easily conjugated on liposomes to obtain EGFR targeted nanocarriers.

Aptamers are short single-stranded DNA or RNA oligonucleotides (6 ~ 26 kDa) that fold into well-defined 3D structures that recognize a variety of biological molecules including transmembrane proteins, sugars and nucleic acids with high affinity and specificity (Phillips et al. 2008, Yan and Levy 2009). A technology called “systematic evolution of ligands by exponential enrichment’ (SELEX)” has been used to identify optimal aptamers for targets on cancer cells (Levy-Nissenbaum et al. 2008, Fang and Tan 2010). Aptamers have been conjugated to nanocarriers for targeted drug delivery. For example, the 2′fluorinated A10 RNA aptamer, which recognizes the extracellular domain of the prostate-specific membrane antigen (PSMA), were conjugated on docetaxel nanoparticles and shown to have high selectivity for tumour and therapeutic efficacy in vivo (Farokhzad et al. 2006).

Peptides (10 ~ 15 amino acids) are able to bind to target proteins, cells and tissues in a specific manner. Furthermore, they are characterized by reduced immunogenicity, high stability, and easy synthesis, scale-up, and chemical conjugations to nanocarriers. Phage display is emerging as the most popular approach (Paschke 2006), which has successfully isolated peptide ligands for somatostatin receptors, hormone receptors (LHRH receptors) (Norberto and Kakar 2009, Sundaram et al. 2009), and markers for the tumour vasculature (Balestrieri and Napoli 2007). In addition to targeting cell surface receptors, effective peptide ligands also have been developed for extracellular matrix (ECM) receptors such as heparin sulphate and hyaluronan (HA), which are overexpressed in tumours (Peer and...
HA targeted liposomes showed significantly improved circulation time and uptake by HA receptor-expressing tumours in vivo (Peer and Margalit 2004a).

**Dual-ligand targeting strategies**

To improve targeting efficiency, dual-ligand directed nanocarriers have been proposed. In biological systems, cell-cell recognition typically involves a multitude of recognition events, e.g., interactions between an antigen presenting cell and a T-cell. Similarly, simultaneous targeting of two receptors on cell surface could lead to greater affinity and specificity for nanocarriers. Indeed, several studies using dual-ligand directed nanocarriers have demonstrated significantly improved therapeutic efficiency in comparison with individual ligand conjugated nanocarriers. For example, Saul et al. (2006) conjugated folic acid and mAb225 to nanocarriers for targeted delivery to KB cells. In another study, Laginha et al. (2005) synthesized dual-Ab nanocarriers by post-insertion of internalizing (anti-CD19) and non-internalizing (anti-CD20) antibodies into liposomes for B-cell targeting. The interaction of CD19 and CD20 antibodies on the same liposome led to synergistic cytotoxicity, by simultaneously engaging two different antigen sites in close proximity on the same cell (Laginha et al. 2005). Dual-peptide nanocarriers against two receptors have also been reported recently (Kluza et al. 2010, Murase et al. 2010).

**Intracellular organelle targeting**

Many molecular drug targets are associated with specific subcellular compartments (Figure 3) (Mukhopadhyay and Weiner 2007, Yousif et al. 2009, Rajendran et al. 2010). Therefore, specifically directing therapeutic agents to an individual organelle is an attractive strategy for drug delivery. Nanocarriers can be designed as efficient delivery vehicles to intracellular organelles, including early-late endosomes, lysosomes, cytoplasm, mitochondria and nucleus. The sequestration of drug nanocarriers within endosomes/lysosomes following endocytosis is one of the most critical bottlenecks for cytoplasmic drug delivery, especially for high molecular weight drugs such as plasmid DNA and siRNAs. Strategies have been designed to increase endosome/lysosome escape through controlled membrane destabilization (e.g., triggered by acidic pH or reducing environment, or proteases). This can be accomplished, e.g., by incorporation of a fusogenic peptide (Kakudo et al. 2004, Futaki et al. 2005). Alternately, nanocarriers can be designed to bypass the endosomal route. This strategy can be achieved by conjugating cell-penetrating peptides to nanocarriers (Brooks et al. 2005, Vives et al. 2008, Zhang et al. 2009). In order to direct drug to mitochondria or the nucleus, specific trafficking signals may be attached on the nanocarriers, e.g., a nuclear localization signal (NLS) (Tkachenko et al. 2003, Misra and Sahoo 2010) and a mitochondrial targeting signal peptide (MTS).

Mitochondrion is a promising therapeutic drug target due to its important role in energy supply and cell death regulation. A variety of methods have been developed to enhance mitochondrial accumulation of drugs (Mukhopadhyay and Weiner 2007, Yamada and Harashima 2008, Yousif et al. 2009). Harashima and Yamada (2008) described a lipid-based carrier multifunctional envelope-type nano-device (MEND) and MITO-Porter. MITO-Porter consists of liposomes conjugated to octaarginine (R8) peptides, which facilitate uptake of the entire assembly into cells by macropinocytosis. Following endosomal escape, MITO-Porter is able to further fuse with the mitochondrial outer membrane due to fusogenic lipids in the liposome (Yamada et al. 2008). The dequalinium (DQA)-based liposomes constitute another class of mitochondrial delivery nanocarriers. DQA is a symmetrical delocalized lipophilic divalent cation, which promotes efficient mitochondrial localization (Weissig et al. 1998, D’Souza et al. 2003). Furthermore, surface modification of liposomes with mitochondriotropic triphenylphosphonium (TPP) cations has been reported to promote the
efficient subcellular delivery of a model drug to mitochondria both in vitro and in vivo (Boddapati et al. 2008).

**Novel delivery strategies for targeted nanocarriers**

**Novel drug nanocarrier compositions**

In parallel to developing novel targeting ligands, novel nanocarrier compositions have also been extensively investigated in the past decade, e.g., minicells (MacDiarmid et al. 2007a, 2007b), apotransferrin (Krishna et al. 2009) and synthetic HDL or LDL lipid nanoparticles (Nikanjam et al. 2007, Thaxton et al. 2009). Moreover, inorganic drug delivery systems such as nanodiamond and single wall carbon nanotube also demonstrated some potential (Bhirde et al. 2009, Lam and Ho 2009). However, among the drug nanocarriers reported, natural and synthetic polymers and lipids remain the dominant materials for constructing drug delivery nanocarriers due to their proven biocompatibility.

MacDiarmid et al. (2007a, 2007b, 2009) described a new type of nanocarrier known as minicells. These are ~400 nm in diameter and are derived from achromosomal bacterial cells. They are immunostimulatory, can stably encapsulate a variety of chemotherapeutics or siRNAs and be specifically targeted using antibodies (MacDiarmid et al. 2007b). Greater tumour growth inhibition was observed with minicell delivery of doxorubicin (DOX) or paclitaxel than with the treatment of ~1875-fold and ~8000-fold higher amounts of their respective free drugs (MacDiarmid et al. 2007a, 2007b).

Nanoparticles made of human serum albumin (HSA) possess several specific advantages including biocompatibility, ease of preparation and covalent modification with targeting ligands. Enrichment of the HSA nanoparticles in tumour tissue may occur by passive or active targeting mechanisms (Hawkins et al. 2008). HAS-based drug delivery has been successfully translated into the clinic. For example, Abraxane, an albumin-nanoparticle formulation of paclitaxel, was approved by FDA in 2005 for the treatment of metastatic breast cancer (Dranitsaris et al. 2009, Miele et al. 2009).

Several other nanocarriers have shown great promises for tumour-targeted delivery. For example, low-density lipoproteins (LDL) or high-density lipoproteins (HDL) have been investigated as nano-carriers for cancer therapeutics due to high LDL/HDL receptor expression on tumours, and their presumed biocompatibility and non-immunogenicity (Nikanjam et al. 2007, Thaxton et al. 2009). Recent studies have shown the feasibility of synthesizing drug loaded LDL/HDL nanoparticles. Zhang et al. (2009, 2010) and Chen et al. (2007) extended the application of synthetic LDL/HDL nanoparticles to cancer diagnostics and therapeutics by conjugating tumour-specific ligands (e.g., folate, EGFR) to reroute LDL/HDL nanoparticles away from their native receptors. Amphiphilic macrocyclic molecules and nanomaterials such as cyclodextrins (CDs) and their derivatives have been investigated as drug nanocarriers based on their ability to encapsulate hydrophobic drugs. Cucurbit[6]uril (CB[6]), a member of the macrocyclic host family cucurbit[n]uril (CB[n]), has a hydrophobic cavity similar to that of ʔCD. Recently, Cucurbituril-based nanoparticles (CB[6] NPs) has been shown as new efficient nanocarriers for delivery of hydrophobic drugs (Park et al. 2009). Furthermore, substantial work has been done with polymeric nanoparticles (Shi et al. 2009a). For example, anti-HER2 conjugated doxorubicin immunonanoparticles enhanced intracellular delivery of the drug to HER2-overespressing SKBR-3 (Shi et al. 2009a).

**Multifunctional nanocarriers**

Recently, multifunctional nanocarriers have attracted much interest (Torchilin 2006a, 2009, Gindy and Prud’homm 2009). These can integrate therapeutic agents, targeting ligands,
imaging agents (e.g., magnetic nanoparticles or quantum dots), cell penetration peptide and stimulus-sensitive components (e.g., pH, temperature or photo-sensitive) into the design of a single nanoparticle. Several recent studies showed that co-delivery of anticancer drugs and MDR targeting siRNA overcame tumour drug resistance. For example, paclitaxel and P-gp targeted siRNA were engineered in the same PLGA-PEI nanoparticles. As a result of silencing of P-gp, paclitaxel delivery efficiency was significantly improved and the tumour drug resistance was overcome both in vitro and in vivo (Patil et al. 2010). Combination of therapeutic drugs and imaging agents (theranostic) has become an important strategy in nanoparticle research. Using imaging agents, the nanoparticles can be tracked in vivo in real-time. Magnetic nanoparticles (MNPs) represent a class of non-invasive imaging agents that can be monitored through magnetic resonance (MR) imaging (McCarthy and Weissleder 2008). MNPs mainly include metallic, bimetallic, and superparamagnetic iron oxide nanoparticles (SPIONs). MNPs have been combined with several anticancer drugs, including paclitaxel, doxorubicin, and methotrexate (MTX) (McCarthy and Weissleder 2008, Dilnawaz et al. 2010). For example, Yang et al. (2007) developed an anti-HER targeted multifunctional magneto-polymeric nanohybrids (HER-MMPNs) for breast cancer, which combined magnetic nanocrystals (for MRI) and the anticancer drug doxorubicin. The resultant HER-MMPNs demonstrated excellent inhibition of tumour growth and ultrasensitive targeted detection by MRI in animal model.

Nanocarriers with stimuli-responsive elements can be triggered to release drug at the tumour sites by the changes in temperature, pH, light, ultrasound, and magnetic field as well as redox potential (Ganta et al. 2008). Nanocarriers (e.g., liposomes, micelles, or polymeric nanoparticles) are able to obtain the stimuli-responsive features via stimuli-sensitive components. For example, Ong et al. (2008), reported stable liposomes (~100 nm in diameter) made from quinone-dioleoyl phosphatidylethanolamine (Q-DOPE) can be triggered by the redox activation of the quinine headgroup to rapidly release their payload.

Concluding remarks

Nanocarriers have played an increasing role in the cancer therapy over the last decade. Compared to free drugs, nanocarrier-encapsulated drugs preferentially accumulate in the tumour sites through the EPR effects, thereby improving therapeutic outcomes and reducing side-effects. Targeting of nanocarrier can further improve the efficiency and specificity of drug delivery. A wide variety of targeted nanocarriers have been developed and demonstrated efficacy in vivo. However, to date there have been no FDA approved targeted nanocarriers and only a handful of them has reached clinical trials. FCE28069, a conjugate of HPMA copolymer, doxorubicin and galactose, is the first receptor-targeted nanoparticle to reach the clinic (Seymour et al. 2002, Duncan 2009). In addition, CALAA-01 (a polymeric nanoparticle containing siRNA), MBP-426 (an oxaliplatin-encapsulated liposome), and SGT-53 (a liposome containing a plasmid DNA against p53 gene) are currently under clinical investigation. All these three kinds of nano-particles are conjugated by a targeting ligand against the transferrin receptor (Heath and Davis 2008). Enormous efforts have been invested in developing novel targeted nanocarriers and the associated therapeutic strategies. The development of complimentary technologies promises to identify more high specificity ligands such as aptamers and peptides. In addition, several novel nanocarriers such as minicells and synthetic LDL/HDL nanoparticles hold great promise for future clinical development. The recent advances in multifunctional targeted nanocarriers further expand the potential of nanocarriers. However, the complexity of novel delivery systems would be substantially increased, which may introduce difficulties in the scale-up production, quality control and gaining regulatory approval. In conclusion, there have been a large volume of work published demonstrating the potential of targeted nanocarriers in...
cancer therapy and relatively few examples of clinical trials of viable products. Future efforts should be focused on clinical translation of novel targeted nanocarriers.

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References


Figure 1.
A schematic diagram representing the accumulation of nanocarriers in tumor sites by passive or active targeting. Both targeted nanocarriers and non-targeted nanocarriers reach tumors selectively through the leaky vasculature in the tumors. Upon arrival at tumor sites, targeted nanocarriers can bind to the target tumor cells or enter the cells via receptor mediated endocytosis.
Figure 2.
Commonly used nanocarriers.
Figure 3.
Schematic illustration of the basic intracellular targets including endolysosomal compartment, mitochondrion, cytoplasm, nucleus for drug, gene and oligo deliveries.
Table I
Commonly used targeting ligands for targeted nanocarriers.

<table>
<thead>
<tr>
<th>Name</th>
<th>Targeting ligand</th>
<th>Receptor</th>
<th>References</th>
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<tbody>
<tr>
<td>Small molecules</td>
<td>Folic acid</td>
<td>Folate receptor</td>
<td>Zhao et al. (2008), Low et al. (2008)</td>
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<td></td>
<td>Galactose</td>
<td>Asialoglycoprotein receptor (ASGPR)</td>
<td>Wu et al. (2002)</td>
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<tr>
<td>Peptides</td>
<td>RGD</td>
<td>αvβ3 integrin receptor</td>
<td>Vives et al. (2008), Garanger et al. (2006)</td>
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<tr>
<td></td>
<td>ATWLPPR (VEGF peptide)</td>
<td>VEGF receptor</td>
<td>Di Benedetto et al. (2008)</td>
</tr>
<tr>
<td>Aptamers</td>
<td>Pegaptanib</td>
<td>VEGF receptor</td>
<td>Ng et al. (2006)</td>
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<td>Proteins</td>
<td>Transferrin</td>
<td>Transferrin receptor</td>
<td>Qian et al. (2002), Daniels et al. (2006)</td>
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<td></td>
<td>Luteinizing hormone releasing</td>
<td>LHRH receptor</td>
<td>Leuschner et al. (2006)</td>
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<td>hormone (LHRH)</td>
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<tr>
<td>Antibodies</td>
<td>Herceptin (Trastuzumab)</td>
<td>Her2/neu (Breast cancer)</td>
<td>Kirpotin et al. (1997), Park et al. (2002)</td>
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<td></td>
<td>Rituxan (Rituximab)</td>
<td>CD20 antigen (B-cell non-Hodgkin lymphoma)</td>
<td>Lapalombella et al. (2008)</td>
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<td></td>
<td>CD19 antibody</td>
<td>CD19 antigen (human B-cell lymphoma)</td>
<td>Lopes de Menezes et al. (1998)</td>
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Table II
Examples of novel ligands for targeted nanocarriers.

<table>
<thead>
<tr>
<th>Name</th>
<th>Targeting ligand</th>
<th>Receptor</th>
<th>Reference</th>
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<tr>
<td>Peptides</td>
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<td>LHDH</td>
<td>aαβ3 integrin</td>
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<td>Norberto and Kakar (2009), Sundaram et al. (2009)</td>
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<td>SP5-2 (TDSILRSYDWTY)</td>
<td>Non-small cell lung cancer</td>
<td>Chang et al. (2009)</td>
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<td>NGR peptide</td>
<td>Angiogenic endothelial cell</td>
<td>Negussie et al. (2010), Pastorino et al. (2003)</td>
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<td>iRGD (CRGDK/RGPD/EC)</td>
<td>Both α5β3 integrin and neuropilin-1 on tumour vessels</td>
<td>Sugahara et al. (2010)</td>
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<td>Aptamers</td>
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<td>A10 RNA aptamer</td>
<td>Prostate-specific membrane antigen</td>
<td>Farokhzad et al. (2006)</td>
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<td>Sgc8c DNA aptamer</td>
<td>Protein tyrosine kinase 7 (PTK7) receptor</td>
<td>Huang et al. (2009)</td>
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<td>Antibodies</td>
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<tr>
<td>Monoclonal antibody A7</td>
<td>Colorectal carcinoma</td>
<td>Toma et al. (2005)</td>
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<td>Transferrin antibody</td>
<td>Transferrin receptor</td>
<td>Gosk et al. (2004)</td>
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<td>DI17E6</td>
<td>α5β3 integrin receptor</td>
<td>Wagner et al. (2010)</td>
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<td>2C5 antibody</td>
<td>Nucleosome (NS)-restricted activity</td>
<td>Torchilin et al. (2003)</td>
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<td>5D4 antibody</td>
<td>Prostate cancer</td>
<td>Sawant et al. (2008)</td>
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<td>Anti-HER2 scFv</td>
<td>ErbB2 receptor</td>
<td>Laginha et al. (2008)</td>
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<td>Anti-VCAM-1</td>
<td>Vascular cell adhesion molecule-1 (VCAM-1) on activated endothelial cells</td>
<td>Voinea et al. (2005)</td>
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<td>Anti-CD22 scFv</td>
<td>CD22 antigen B-cell lymphomas</td>
<td>Loomis et al. (2010)</td>
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<td>Other targeting molecules</td>
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<td>Affibody</td>
<td>EGFR receptor</td>
<td>Beutler et al. (2009)</td>
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<td>Avimer</td>
<td>Human extracellular receptor</td>
<td>Silverman et al. (2005)</td>
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<td>Nanobody</td>
<td>Human tumour-associated carcinoembryonic antigen</td>
<td>Cortez-Retamozo et al. (2004)</td>
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