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Strategies of polymeric nanoparticles for enhanced internalization in cancer therapy

Lu Sun, Qinjie Wu, Feng Peng, Lei Liu, Changyang Gong*

Department of Medical Oncology, Cancer Center, State Key Laboratory of Biotherapy/Collaborative Innovation Center of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, PR China

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ABSTRACT

In order to achieve long circulation time and high drug accumulation in the tumor sites *via* the EPR effects, anticancer drugs have to be protected by non-fouling polymers such as poly(ethylene glycol) (PEG), poly(ethylene oxide) (PEO), dextran, and poly(acrylic acid) (PAA). However, the dense layer of stealth polymer also prohibits efficient uptake of anticancer drugs by target cancer cells. For cancer therapy, it is often more desirable to accomplish rapid cellular uptake after anticancer drugs arriving at the pathological site, which could on one hand maximize the therapeutic efficacy and on the other hand reduce probability of drug resistance in cells. In this review, special attention will be focused on the recent potential strategies that can enable drug-loaded polymeric nanoparticles to rapidly recognize cancer cells, leading to enhanced internalization.

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1. Introduction

Chemotherapeutic drugs generally suffer from poor pharmacokinetics and inappropriate biodistribution. Because of their low molecular weight (Mw), for instance, intravenously (*i.v.*) administered anticancer agents tend to present with short circulation time and with low concentrations in tumors and metastases. To assist *i.v.* administered anticancer agents in achieving proper circulation time and tumor concentration, and to attenuate their accumulation in potentially endangered healthy organs and tissues, nanoscale drug delivery systems such as liposomes, polymeric micelles, polymersomes, nanogels, and nanocapsules have emerged as an indispensable platform for modern cancer therapy [1–3]. Their appropriate sizes (usually between several nanometers and 200 nm) and stealthy properties enable them to extravagate through the hyperpermeable blood vessels and preferentially accumulate in the tumor *via* the enhanced permeability and retention (EPR) effect [4–6]. Since the circulation time of a carrier is

* Corresponding author.
E-mail address: chygong14@163.com (C. Gong).

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prolonged, its opportunity of passing through the leaky vasculature increases, and thereby its extravagation into the tumor tissue [7]. Beyond that, therapeutic polymeric nanoparticles (NPs) can accommodate multiple functions including: (1) improve the pharmaceutical and pharmacological properties of drugs, potentially without the need to alter drug molecules, (2) deliver multiple types of therapeutic drugs with potentially different physicochemical properties, (3) deliver a combination of imaging and therapeutic agents for real-time monitoring of therapeutic efficacy, (4) protect drugs (small molecules, proteins, nucleic acids or peptides) from hepatic inactivation, enzymatic degradation and rapid clearance *in vivo*, (5) reduce the incidence and intensity of side effects [8]. Hence, numerous studies focused on NPs with antineoplastic drugs encapsulated in during the past decades. And a number of drug-loaded NPs have reached clinical development and even some have been clinically approved. For instance, DOXIL, doxorubicin (Dox)-loaded PEGylated liposome, was the first FDA approved liposome nanomedicine to reach clinical approval in 1995 for AIDS related Kaposi's syndrome [9]. NK911, a micellar NP comprising PEG, Dox and poly(aspartic acid), and Genexol-PM, which was a paclitaxel-encapsulated PEG-PLA micelle formulation, both were currently in phase II development for various cancers [10–12].

Thus far however, the clinical performance of EPR-exploiting drug-loaded NPs has been relatively disappointing. They do substantially reduce the incidence and intensity of side effects, such as cardiotoxicity, bone marrow depression, alopecia and nausea, but to date, they have largely failed to really improve response rates and survival times [13,14]. The majority of drug-loaded polymeric nanoparticles possess a stealth surface made of water soluble non-fouling polymers such as poly(ethylene glycol) (PEG), poly(ethylene oxide) (PEO), dextran, and poly(acrylic acid) (PAA), which confer prolonged circulation time and enhanced accumulation in tumor sites *via* EPR effect [15,16]. However, this highly hydrophilic surface failed to create optimal uptake by cancer cells within the tumor. This problem which has been referred to by some as the "PEG dilemma" has been suggested to hinder efficient drug delivery in tumors as these NPs end up releasing their therapeutic payload into the tumor milieu rather than within cancer cells [17,18]. And ubiquitously targeting cells within a tumor is not always feasible because some drugs cannot diffuse efficiently and the random nature of the approach makes it difficult to control the process of internalization. This lack of control may induce multiple-drug resistance (MDR)-a situation where chemotherapy treatments fail patients due to resistance of cancer cells toward one or more drugs. MDR occurs because transporter proteins that are overexpressed on the surface of cancer cell can expel drugs from cells [19–21]. Expelling drugs inevitably lowers the therapeutic effect and cancer cells soon develop resistance to a variety of drugs. Consequently, as vehicles, ideal nanoparticles are obliged to target cells with high drug loading levels without drug leakage on the way, while rapidly unload drug at the intracellular site of action. In this review, special attention will be focused on the recent potential strategies that can enable drug-loaded polymeric nanoparticles to rapidly recognize cancer cells, leading to enhanced internalization.

2. Ligand-targeted NPs for enhanced internalization

The addition of targeting ligands, which was installed on the surface of nanoparticles, can play a vital role in the ultimate location of the nanoparticle. For example, nanoparticles can be selectively recognized by specific tumor cells if their surfaces contain moieties such as antibodies, aptamers, proteins, peptides, folate, carbohydrate and other emerging targeting molecules. These moieties can be directed to cancer cell surface receptors, such as transferrin receptors, that are known to be increased in number on a wide range of cancer cells [22]. These targeting ligands enable nanoparticles to bind to cell-surface receptors and enter cells *via* the receptor-mediated endocytic route. Recent work comparing non-targeted and targeted nanoparticles (lipid-based [23] or polymer-based [24]) has demonstrated that the primary role of the targeting ligands is to enrich cellular uptake into cancer cells rather than to increase the accumulation in the tumor. In the following section we mainly focus on recent efforts in the development of ligand-based targeted NPs (Table 1).

2.1. Monoclonal antibody based targeting molecules

Among all the targeting molecules, monoclonal antibodies (mAbs) have been most commonly used in the development of targeted NPs owing to their high specificity and affinity to the target and so far about 30 of them have been approved for clinical use [44–48]. For example, trastuzumab and rituximab, which are mAbs currently in the clinic, have been conjugated to poly(lactic acid) (PLA) NPs leading to nanoconjugates that demonstrate a 6-fold increase in the rate of particle uptake compared with similar particles lacking mAb targeting molecules [49,50]. A nanoparticle consisting of a mucic acid polymer conjugate of camptothecin (CPT), MAP-CPT, and containing herceptin antibody was investigated in bearing HER2 overexpressing BT-474 human breast cancer cells. Cellular uptake of nanoparticles was enhanced by 70% compared to nontargeted version by the incorporation of a single Herceptin antibody targeting agent per nanoparticle [51,52]. Gold nanoparticles (AuNPs) were conjugated with cetuximab (C225) and then labeled with In-111, which created EGFR-targeted AuNPs. *In vitro* studies showed that the uptake of C225-conjugated AuNPs in high EGFR-expression A549 cells was 14.9-fold higher than that of PEGylated AuNPs; moreover, uptake was also higher at 3.8-fold when MCF7 cells with lower EGFR-expression were used. *In vivo* A549 tumor xenograft mouse model MicroSPECT/CT imaging and a biodistribution study provided evidence of enhanced internalization of the C225-conjugated AuNPs into the tumor cells *via* antibody-mediated endocytosis. But a large portion of PEGylated AuNPs remained in the tumor interstitium [53]. Despite the intense effort undertaken for their development, mAbs-conjugated NPs still encounter many challenges and limitations. First, mAbs-conjugated NPs have a large size, which curbs intratumoral distribution due to interstitial tumor pressure and limits their intracellular and intratissue penetration especially in solid tumors. Second, they require extensive optimization through molecular engineering technologies, and create engineering difficulty in NPs scale-up and manufacturing. Third, they potentially lead to increased immunogenicity – the ability to evoke an immune response – and liver and spleen uptake of the nanocarrier [54–56]. For these reasons Abs can be fragmented and only the antigen-binding fragments are used. It is true that for better treatment, the faster speed of penetration in solid tumors of antibody fragments over intact antibodies is a remarkably superiority [45]. Additionally, although antibody fragments including antigen-binding fragments (Fab), dimers of antigen-binding fragments ($F(ab)_2$), single-chain fragment variables (scFv) and other engineered fragments (Fig. 1) are less stable than whole antibodies, they are considered safer when injected systemically due to reduced non-specific binding [55]. Two antibody fragment targeting liposomal systems have progressed to clinical trials. MCC-465 is an immunoliposome-encapsulated doxorubicin (Dox), with a surface decorated with both PEG and dimers of antigen-binding fragments ($F(ab)_2$) for immune shielding and targeting respectively. The $F(ab)_2$ used in this NPs is a fragment of the human mAb, GAH, which positively reacts to >90% of cancerous stomach tissues, but negatively to all normal tissues [57]. MCC-456 exhibits significant antitumor response against GAH-positive xenografts leading

Table 1
Examples of Ligand-Directed Targeting Polymeric Nanoparticles for Cancer Treatment.

Identity	Ligand type	Target	Nanoparticle	Cargo	Target Cells/Tissues	Rf
herceptin	antibody	HER2[a]	polymeric nanoparticle	tamoxifen	breast cancer	[25]
Cetuximab (C255)	antibody	EGFR[b]	Nanographene oxide	epirubicin	glioma cells	[26]
anti-Tf[c] Fab′	antibody fragment	TfR[d]	micelle	DACHPt/m	pancreatic cancer	[27]
HAb18 F(ab′)(2)	antibody fragment	tumor antigen	micelle	doxorubicin	hepatocellular carcinoma	[28]
Z_{HER2} affibody	affibody	HER2	bio-nanocapsule/liposome complex	siRNA	breast cancer cells	[29]
EGa1	nanobody	EGFR	micelle	doxorubicin	head and neck squamous cell carcinoma cells	[30]
AS1411	aptamer	nucleolin	polymeric nanoparticle	vinorelbine	breast cancer cells	[31]
RNA aptamer	aptamer	PSMA[e]	liposome	doxorubicin	prostate cancer	[32]
Tf	protein	TfR	polymeric nanoparticle	microRNA2-9b	acute myeloid leukemia	[33]
Lactoferrin	protein	LRP[f]	BSA nanoparticle	doxorubicin	glioblastoma	[34]
RGD	peptide	$\alpha_v\beta_3$ integrin	dendrimer	doxorubicin	glioblastoma	[35]
cRGD	peptide	$\alpha_v\beta_3$ integrin	micelle	platinum	glioblastoma	[36]
iRGD	peptide	αv integrin	polymeric nanoparticle	paclitaxel and surviving shRNA	lung cancer	[37]
LyP-1	peptide	p32/gC1q	liposome	doxorubicin	lymphatic metastatic tumor	[38]
FA[g]	FA	FRs[h]	hydrogel	curcumin	HeLa cells	[39]
FA	FA	FRs	polymersome	doxorubicin	glioma cells	[40]
Galactose	Carbohydrate	ASGPR[i]	polymersome	protein	hepatoma cells	[41]
HA[j]	Carbohydrate	CD44	polymer nanoparticle	paclitaxel	SCC7 cells	[42]
BIND-014	Small molecule	PSMA	polymer nanoparticle	detaxel	solid tumors	[43]

[a] Human epidermal growth factor receptor 2.
[b] Epidermal growth factor receptor.
[c] Transferrin.
[d] Transferrin receptor.
[e] Prostate specific embrane antigen.
[f] Low-density lipoprotein receptor.
[g] Folic acid.
[h] Folate receptors.
[i] Asialoglycoprotein receptor.
[j] Hyaluronic acid.

to up to 80% reduction in tumor mass compared with free Dox or GAH non-conjugated PEG liposomal Dox. However, MCC-465 does not appear to have progressed through clinical development after phase I completion [58]. SGT-53 is a therapeutic complex that comprises of a cationic liposome encapsulating a plasmid encoding normal human wild-type p53 DNA, and is decorated with an anti-transferrin receptor (TfR) single-chain antibody fragment (scFv) designed to target cancer cells. Pre-clinical studies have indicated that SGT53-01 could sensitize tumors to the effects of radiation and chemotherapy. SGT-53 is currently in a Phase Ib clinical trial (2007, NCT00470613) in patients with advanced solid tumors [59]. The anti-CD44v6 single chain variable fragment (scFv(CD44v6)) screened out from the human phage-displayed scFv library possesses high specificity and affinity to membrane antigen CD44v6 overexpressing in a subset of epithelium-derived cancers, such as pancreatic, hepatocellular, colorectal and gastric cancers. Conjugation of scFv(CD44v6) with arsenite ion (As) loaded polymeric nanoparticles (scFv-As-NPs) enabled more efficient delivery of As and exhibited higher cytotoxic activity than non-targeted ones (As-NPs) in human pancreatic cancer cells PANC-1. Furthermore, the targeted delivery of As induced more significant gene suppression in terms of the expression of anti-apoptotic Bcl-2 protein [60].

Affibody constitute a new class of targeted moiety based on an antibody binding domain of staphylococcal protein A [61]. Due to small size (6 kDa) as well as high affinity and specificity, affibody molecules are well suited to provide small particles with affinity-mediated recognition for cellular targets [62]. Affibody molecule has been reported with selective binding to therapeutic targets with subnanomolar affinity-for example, human epidermal growth factor receptor 2, epidermal growth factor receptor, insulinlike growth factor 1, and platelet-derived growth factor receptor β [63–65]. Julia Beuttler et al. applied a bivalent, high-affinity EGFR-specific affibody molecule for the generation of targeted PEGylated liposomes. Compared to non-targeted liposomes, targeting led to internalization in EGFR-expressing cell lines and a selective increase in cytotoxicity toward EGFR-expressing tumor cells when using mitoxantrone loaded liposomes [66]. In addition, affibody modified and radiolabeled gold-iron oxide hetero-nanostructures were developed for tumor PET, optical and MR imaging. *In vitro* and *in vivo* study showed that the resultant nanoprobe modified with targeting molecules (anti-EGFR Affibody protein) provided high specificity, sensitivity, and excellent tumor contrast for both PET and MRI imaging in the human EGFR-expressing cells and tumors [67].

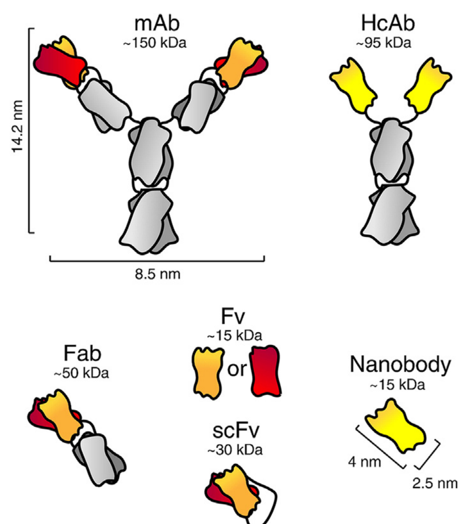

Fig. 1. Antibodies and their fragments. Schematic representation and corresponding molecular weight of (left) a monoclonal antibody, mAb, and its fragments, *i.e.*, Fab′, Fv, scFv; and of (right) a heavy chain only antibody, HcAb, together with its antigenbinding fragment, *i.e.* nanobody.

Source: Adapted from Ref. [70] with kind permission of P.M. van Bergen en Henegouwen, Division of Cell Biology, Department of Biology, Faculty of Science, Utrecht University, The Netherlands.

The variable domain of the heavy-chain antibodies, which is the smallest fully functional antigen-binding fragment, we refer to this entity as nanobody [68]. Nanobodies, with a molecular weight of 15 kDa and 1.5–2.5 nm dimensions, combine the advantages of both small molecules (*e.g.*, molecular cavity binding, low production costs) and monoclonal antibodies (*e.g.*, high affinity and specificity) [69,70]. In addition, these smaller molecules have been shown with lower immunogenicity than the traditional mAbs [71,72]. Isil Altintas et al. have published the successful development of novel targeted albumin nanoparticles, *i.e.* the nanobody-albumin nanoparticles (NANAPs). The surface of albumin nanoparticles was coated with bifunctional polyethylene glycol 3500 (PEG) and a nanobody-the single variable domain of an antibody-(Ega1), to provide specificity for binding to EGFR-expressing tumor cells. These nanoparticles were loaded with a multikinase inhibitor 17864-Lx-a platinum-bound sunitinib analog, which couples the drug to methionine residues of albumin and is released in a reductive environment. These NANAPs were efficiently internalized into the 14C squamous head and neck cancer cells *via* the interaction of the nanobody with EGFR. EGa1-PEG functionalized nanoparticles showed a 40-fold higher binding to EGFR-positive 14C cancer cells in comparison to PEGylated nanoparticles. The intracellular routing of EGa1 targeted nanoparticles leads to a successful intracellular release of the kinase inhibitor and inhibition of proliferation whereas the non-targeted formulations had no antiproliferative effects on 14C cells [73].

2.2. Aptamer-based targeting molecules

In addition to the rational design of antibodies, highthroughput approaches have been used to generate targeting agents such as aptamers, which are single-stranded DNA or RNA oligonucleotides selected *in vitro* from a large number of random sequences ($\sim10^{14}$–10^{15}) [55,74]. The *in vitro* aptamers selection process is referred to as Systemic Evolution of Ligands by Exponential enrichment (SELEX) [75] introduced in the early 90s independently by the research groups of Gold [76] and Ellington [77]. Aptamers are selected to bind to a wide variety of targets with high specificity and affinity, including proteins, phospholipids, iron channels, nucleic acids, and whole cells, among others [78]. Compared with traditional antibodies, nucleic acid aptamers show significant advantages in terms of size, non-immunogenicity, remarkable stability in a wide range of pH (4–9) and temperature, as well as to rare activity loss when exposed to organic solvents [79]. In addition, structural flexibility enables aptamer to bind to hidden epitopes which cannot be targeted by antibodies [80]. Furthermore, nucleic acid ligands identified through the SELEX process can be obtained in a large amount through chemical synthesis, which is cost-effective and has minimal batch to batch difference in binding affinity. This is in contrast to antibodies, which often display batch-to-batch variation in quality during scale-up production [81,82].

Since first reported, more than 200 kinds of aptamers have been isolated for a wide variety of targets [83,84]. For example, RNA aptamers to the vascular endothelial growth factor ($VEGF_{165}$) isoform with 2′-O-methylpurine and 2′-F pyrimidines, were isolated by NeXstar Pharmaceutical (acquired by Gilead Science in 1999) in 1995 and 1998, respectively. VEGF aptamers could not only contribute to regression of tumor vessels, but also illustrate a remarkable stability in plasma in monkeys [85]. The approval of $VEGF_{165}$-targeted Macugen (Pegaptanib sodium) by the FDA in 2004 for the treatment of neovascular macular degeneration, underscored the rapid progress of aptamers from original conception to clinical application [86]. By integrating the advantages of NPs with the cell-targeting capabilities of aptamers, aptamer-functionalized NPs may provide a new path of designing solutions for the drug delivery field [78,87–89]. AS1411, an aptamer showing specific binding to nucleolin, was conjugated to PEGylated cationic liposome as the targeting probe ASLP (AS1411-PEG-liposome) for siRNA delivery. The much higher accumulation of the siRNA in tumor cells comparing with normal cells indicated that ASLP displayed excellent tumor-targeting capability. Notably, ASLP/siBraf showed significant silencing activity in A375 tumor xenograft mice and inhibited the melanoma growth [90]. In one preliminary study, platinum compound (PtIV)-encapsulated NPs formulated with PLGA-b-PEG copolymer was constructed where the surface was functionalized with A10 aptamers. A10 aptamers, first isolated in 2002, could recognize ta hallmark antigen on prostate cancer cell surfaces, named prostate specific membrane antigen (PSMA). PSMA is predominantly expressed on the vasculature of many neoplasms but lowly expressed in normal tissue [91]. These Pt(IV)-encapsulated nanoparticle-aptamer bioconjugates (Pt-NP-Apt) bind to the PSMA protein expressed on the surface of LNCaP prostate epithelial cells and get taken up by these cells resulting in significant enhancement in *in vitro* cellular toxicity as compared with nontargeted nanoparticles that lack PSMA aptamer (Pt-NP). And enhanced *in vivo* pharmacokinetics, biodistribution, tolerability, and efficacy of Pt-PLGA-b-PEG-Apt-NP when compared to cisplatin administered in its conventional form was demonstrated in normal Sprague Dawley rats, Swiss Albino mice, and the PSMA-expressing LNCaP subcutaneous xenograft mouse model of PCa, respectively [92]. When the combination delivery system was functionalized with aptamer, the resultant vehicle delivering different drugs is anticipated to be a more effective disease targeting system. In one study, A10-functionalized, dual-drug delivery NP platform was designed, allowing for simultaneous co-delivery of hydrophobic docetaxel and hydrophilic PtIV. This powerful carrier exhibited 10-fold increased toxic efficacy compared with a single PtIV carrier [93].

In fact, following insight into novel tumor biomarkers, other new aptamers are still under selection and investigation. For example, a novel hollow gold nanosphere (HAuNS) drug delivery system was loaded with Dox for killing tumor cells and equipped with an aptamer (Apt) for selective targeting CD30 (a cell membrane protein of the tumor necrosis factor receptor family) on lymphoma cells [94]. Curcumin-loaded lipid-polymer-lecithin hybrid

nanoparticles were functionalized with RNA aptamers against epithelial cell adhesion molecule (EpCAM) for targeted delivery to colorectal adenocarcinoma cells. These Curcumin-encapsulated bioconjugates (Apt-Cur-NPs) showed increased binding to HT29 colon cancer cells and improvement in cellular uptake when compared to Cur-NPs functionalized with a control Apt. And a substantial enhancement in cytotoxicity was achieved toward HT29 cells with Apt-Cur-NP bioconjugates [95]. GMT8 aptamer, a short DNA sequence that could specifically bind to glioblastoma U87 cells, was used to functionalize docetaxel-loaded PEG-PCL nanoparticles. *In vitro* cell uptake and U87 tumor spheroid uptake study demonstrated that nanoparticles functionalized with GMT8 aptamer (ApNP) significantly enhanced intracellular drug delivery and tumor spheroid penetration. The *in vivo* imaging demonstrated that in an orthotropic brain glioblastoma model, ApNP could target glioblastoma resulting in 2-fold higher tumor accumulation than nontargeted controls [96].

Although aptamers have many advantages over antibodies, they are still challenged by several *in vivo* barriers. First, since most aptamers are obtained from *in vitro* selection under a simplified environment, they may lose specificity when applied *in vivo* when the environment becomes more complex. Second, aptamers may suffer degradation in blood circulation by the serum nuclease. Thus, an aptamer agent may be cleared/degraded before reaching the tumor site. Third, its polar nature may prevent it from sufficiently penetrating the tumor. If the issues at the design stage are resolved, aptamer-functionalized NPs may have a higher chance for clinical translation.

2.3. Protein and peptide-based targeting molecules

Many endogenous proteins that selectively bind to specific membrane receptors have been proposed to achieve effective tumor targeting *via* receptor-mediated endocytosis. For example, the iron transporting protein Tf, which binds specifically to the TfR, has been exploited to deliver NPs into different cell types. Choi et al. found that ligands targeting the TfR exerted their influence by increasing uptake of targeted NPs by cancer cells while not by increasing particle accumulation in the tumor region [97]. Active targeting of brain malignancies could be also achieved by Tf-conjugated nanoplatforms, as this molecule could facilitate the transcytosis of drug loaded colloids across the blood brain barrier [98]. The Tf targeting nanoparticle formulation of siRNA, denoted as CALAA-01, was the first RNA interference (RNAi)-based, experimental therapeutic to be administered to cancer patients [99,100]. MBP-426, a liposome conjugated with Tf for the delivery of oxaliplatin, has completed a Phase I clinical trial (2006, NCT00355888). It has also reached a Phase Ib/II clinical trial in second-line patients with gastric, gastroesophageal, or esophageal adenocarcinoma (2009, NCT00964080) [101]. Lactoferrin (Lf) is another iron-binding protein which could adhere to low-density lipoprotein receptor (LRP) overexpressed in the glioma cells. Lf has been explored as a specific targeting molecule to promote the delivery of anticancer drugs to brain [102]. Bovine serum albumin nanoparticles (BSA-NPs) modified with both Lf and mPEG2000 loading Dox was designed, and its brain glioma cells targeting properties were investigated. Compared to other NPs, Lf-NPs showed the strongest cytotoxicity and the highest effectiveness in the uptake both in the primary brain capillary endothelial cells (BCECs) and glioma cells (C6). Body distribution of Dox in different formulations revealed that Lf-NPs could significantly increase the accumulation of Dox in the brain [34]. Alternatively, growth factor interactions with cancer cells represent a commonly used targeting strategy, as cancer cells often overexpress the receptors for nutrition to maintain their fast-growing metabolism. For example, epidermal growth factor (EGF) has been shown to block and reduce tumor expression of the EGF receptor, which is overexpressed in a variety of tumor cells such as breast and tongue cancer [103]. Direct coupling of EGF to nanocarriers containing chemotherapies such as drugs has improved intracellular delivery and therapeutic outcome in animal tumor models [104]. Neural growth factor (NGF) attached to NPs also have been shown to enter cells and elicit specific molecular responses [105]. However, proteins are often immunogenic and susceptible to early clearance by different mechanisms *in vivo*, which may largely limit their effectiveness. In addition, receptors of these protein ligands are endogenous and commonly expressed on fast-growing healthy cells such as firoblasts, epithelial and endothelial cells. This could lead to non-specific targeting and subsequently decrease drug effectiveness and increase toxicity [106].

Peptides are particularly well suited for NPs targeting due to their small size, relatively low immunogenicity as compared to larger proteins, high stability, and ease of conjugation to NPs surfaces. Additionally, the multivalent presentation of a peptide on NPs provides high avidity for the target [107]. Specifically, the development of highly specific peptide phage libraries, bacterial peptide display libraries, plasmid peptide libraries, and new screening technologies have made it possible to synthesize peptide ligands to a myriad of targets [108,109]. Combinatorial libraries have contributed to the discovery of short peptides (10–15 amino acids) that are capable of binding to targeted proteins, cells, or tissues specifically [110]. The integrin family of cell adhesion receptors regulates a diverse array of cellular functions crucial to the initiation, progression and metastasis of solid tumors. However, integrin expression can also vary considerably between normal and tumor tissue. Most notably, $\alpha_v\beta_3$ integrin is usually expressed at low or undetectable levels in most adult epithelia but can be highly upregulated on tumoral endothelial cells as well as on some tumor cells [111]. A search for high affinity ligands for integrin receptors, Arg-Gly-Asp (RGD) peptides are well-known to bind preferentially to the $\alpha_v\beta_3$ integrin [112]. A cyclic RGD peptide antagonist of $\alpha_v\beta_3$, cilengitide (EMD121974), showed favorable safety profiles and no-dose-limiting toxicities in phase I clinical trials [113]. Cilengitide is currently being tested in phase II trials in patients with lung and prostate cancer [114] and glioblastomas [115,116]. RGD-targeted nanocarriers may specifically address drugs to angiogenic endothelial cells and/or cancer cells by interaction the RGD peptide with $\alpha_v\beta_3$ overexpressed by these cells, allowing “double active targeting” of the tumors [36,117,118]. As illustrated by Fig. 2, the EPR effect combined with the double active targeting of both endothelial and cancer cells leads to the antitumoral effect of drug-loaded RGD nanocarriers. N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymer-docetaxel-RGDfK conjugate was synthesized and evaluated *in vitro* and *in vivo* in comparison with untargeted HPMA copolymer-docetaxel conjugates. The RGDfK targeted conjugate showed active binding to αvβ3 integrins in both human umbilical vein endothelial cells (HUVEC) and DU145 human prostate cancer cells, whereas the untargeted conjugate demonstrated no evidence of specific binding. And RGDfK targeted conjugates exhibited the highest antitumor efficacy as evaluated by tumor regression compared to nontargeted HPMA-DTX counterparts in mice bearing DU145 human prostate tumor xenografts [119]. Paclitaxel (PTX) and $cRGDfK_2$ conjugated poly(glutamic acid) (PGA-PTX-E-($cRGDfK_2$)) could inhibit the growth of proliferating αvβ3-expressing HUVEC, U87MG human glioblastoma, and 4T1 murine breast cancer [120]. Dox-loaded cRGDfk-functionalized PEO-PCL micelles showed high affinity with bladder cancer cells (T-24 cells) and strong inhibitory effect on their proliferation [121]. It was worth noting that cRGDyk conjugated liposomes could serve as an effective drug system for targeted and synergistic therapy of bone metastases from prostate cancer. By taking advantages of targeted drug delivery and synergistic antitumor activity of cRGDyk

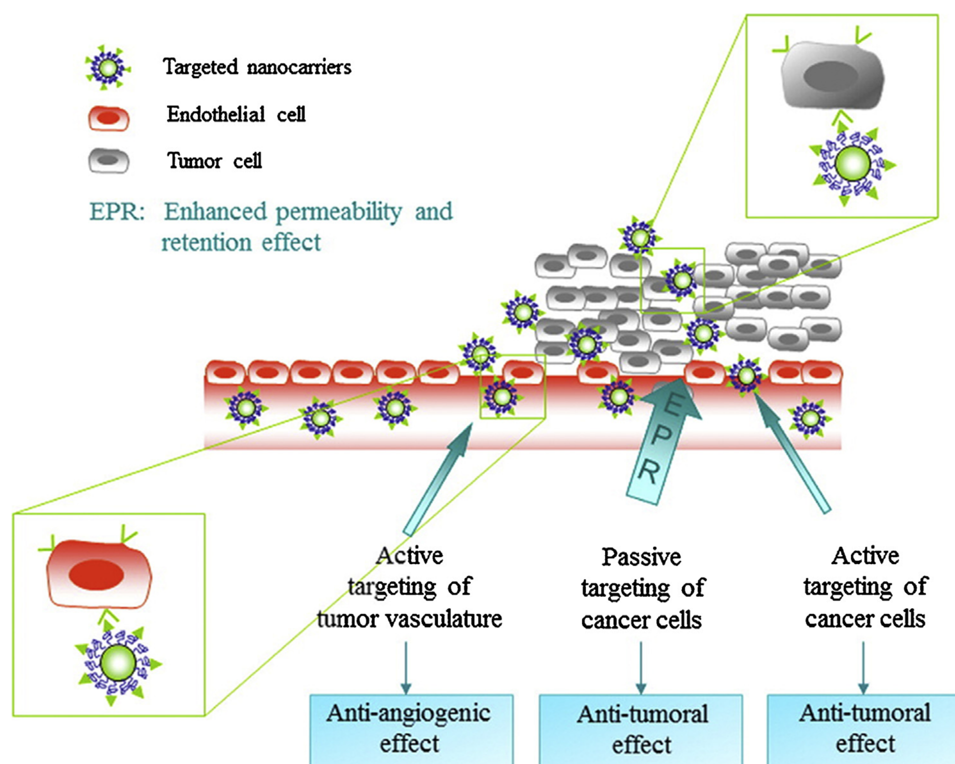

Fig. 2. Schematic representation of targeting mechanisms of RGDgrafted nanocarriers.
Source: Adapted from Ref. [221] with kind permission of V. Preat, Université catholique de Louvain, Pharmaceutics and Drug Delivery, Louvain Drug Research Institute, Belgium.

and loaded cisplatin, cRGDyk conjugated liposomal drug system could inhibit osteoclastic and osteoblastic bone lesions, relieve pain, and improve overall survival [122].

To further retain NPs within tumor tissue and to enhance cellular uptake, cell-penetrating peptides have been used to facilitate translocation of cargoes across the plasma membrane and to specific organelles within the cell [123]. The cyclic iRGD is constituted from CRGDK/RGPD, containing a cryptic CendR motif, CRGDK/R that possesses CendR-like tissue and cell penetrating activities [124]. Like conventional RGD peptides, iRGD homes to tumors by initially binding to α_v integrins that are expressed on tumor blood vessel endothelial cells and tumor cells [110,125]. iRGD is then proteolytically cleaved in the tumor to produce CRGDK/R. The truncated peptide loses much of its integrin-binding activity, but gains affinity for neuropilin-1 (NRP-1) [126]. The NRP-1 binding triggers extravasation, tissue penetration, and cell entry of NPs, which is tumor-specific because the cleavage requires earlier binding of the peptide to integrins (Fig. 3). Interestingly, the recent papers have shown that iRGD-decorated PTX-loaded micelles with a long poly(N-vinylpyrrolidone) block exhibited superior tumor growth inhibition and survival time of H22 tumor-bearing mice [127]. And the recent findings strongly suggested that iRGD-mediated drug delivery system could significantly improve the efficacy of tumor therapy through enhancing tumor accumulation and penetration as compared to the conventional RGD ones. The RGD cyclopeptide (RGDyC) or iRGD was bound to PEGylated polyamidoamine (PAMAM) dendrimer with Dox by acid-sensitive cis-aconityl linkage (PEG-PAMAM-cis-aconityl-Dox, PPCD), respectively. *In vitro* glioma spheroid penetration study showed that RGD-PPCD, iRGD-PPCD and iRGD + PPCD penetrated into C6 spheroids with a depth of 115 μm, 144 μm and 150 μm, respectively, indicating that iRGD-mediated PPCD delivery system had a stronger penetrating ability than RGD ones. *In vivo* results also demonstrated the superiority in penetration ability and accumulation in brain tumor of iRGD system

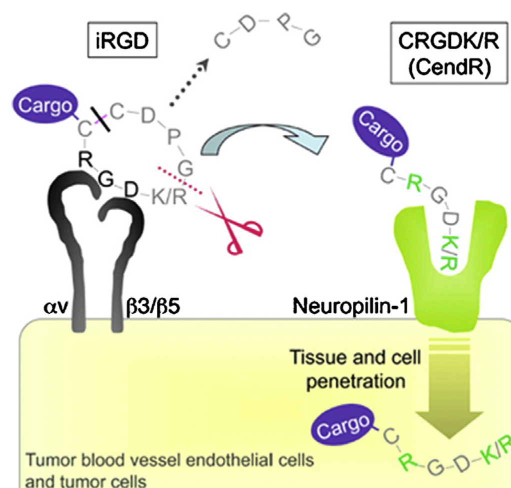

Fig. 3. Tissue penetrating iRGD. Penetration mechanism of iRGD. The iRGD peptide binds to α_v integrin expressed by tumor blood vessel endothelial cells and tumor cells. After the binding, the peptide is cleaved by proteases to expose the cryptic CendR element. This CendR element binds to neuropilin-1 and penetrates into cells and tissue. The peptide can also penetrate into tumors while carrying a cargo attached to the N-terminus of the iRGD peptide.
Source: Adapted from Ref. [221] with kind permission of V. Preat, Université catholique de Louvain, Pharmaceutics and Drug Delivery, Louvain Drug Research Institute, Belgium.

over RGD ones [128]. LyP-1 (Cys-Gly-Asn-Lys-Arg-Thr-Arg-Gly-Cys), a cyclic nine-amino acid peptide identified by *in vivo* phage display technology, was proved to be able to specifically recognize and bind to its receptor (p32/gC1qR) in certain tumor cells (such as highly metastatic breast tumor cells MDA-MB-435S and MDA-MB-231), offering the potential for a drug delivery system to selectively target tumor [129,130]. LyP-1 modified nanoparticles have been

reported for their good tumor targeting and enhanced inhibition effect of drugs loaded against tumors [131]. Besides, LyP-1 peptide was found to specifically bind with tumor lymphatic vessels, providing one possible avenue for tumor targeted therapy that can specifically destroy tumor lymph system simultaneously [132]. To specifically deliver drug to both highly metastatic tumor and its lymphatics, tumor- and tumor lymphatics-homing peptide (LyP-1) conjugated PEG-PCL micelles (LyP-1-PM) were first constructed. The attachment of LyP-1 peptide on the surface of polymeric micelles indeed led to their favorable uptake in highly metastatic breast cancer MDA-MB-435S cells and lymphatic endothelial cells (LEC) *in vitro* and their specific delivery to the tumor and tumor lymphatics *in vivo* [133]. F3 is a 31-amino acid sequence of the NH2-terminal fragment of human high-mobility group protein 2, which was discovered using phage-displayed cDNA libraries [134]. The 31-amino-acid F3 peptide has also been shown to bind to nucleolin protein expressed on the surface of tumor endothelial cells and tumor cells [135]. F3-targeted cisplatin-hydrogel nanoparticles (F3-Cis-Np) bind with high specificity to both human ovarian tumor cells and tumor endothelial cells *in vitro*, and *in vivo*. F3-Cis-Np led to near complete loss of all human tumor vessels in a murine model of human tumor vasculature [136].

Hence, results up to now suggest the accumulation of anticancer drugs in tumors can be appropriately enhanced by peptide-functionalized nanoparticles when compared to non-targeting nanoparticles. But overall delivery to the tumor remains typically low, and there is substantial delivery to other ‘healthy’ tissues like the liver and spleen. In addition, the capability of the peptide-functionalized nanoparticles to show similarly enhanced cell targeting when injected *in vivo* is complicated by the heterogeneity of cells within the tumor microenvironment and the variable expression levels of the target proteins. Nonetheless, these issues do not negate the benefits obtained by the peptide-functionalized nanoparticles currently used to improve cell binding and internalization, but rather indicate that the nanoparticles face additional challenges when used for *in vivo* therapy [137].

2.4. Folate-based targeting molecules

One of the most extensively studied small molecule targeting moieties for drug delivery is folic acid (FA). FA is necessary for the synthesis of purines and pyrimidines. FA binds with a high affinity (in the nanomolar range) to the glycosylphosphatidylinositol-linked folate receptors (FRs), which is overexpressed in the cancerous cells as compared to the normal cells. The expression level of FA in tumors have been reported to be 100–300 times higher than that in normal tissues [138]. This speciality conferred a variety of FA derivatives and conjugates to deliver molecular complexes to cancer cells without doing harm to normal cells [139]. Furthermore, FA is much easier to be manufactured at an industrial scale compared with antibodies, which is an apparent merit of FA targeting for clinical application [140]. For example, EC145 is conjugate of FA and a vinca alkaloid (desacetylvinblastine monohydrazide (DAVLBH)) currently in phase III development for cancer treatment [141,142]. Based on the natural high affinity of FA for the FRs, drug carriers conjugated with FA may also bind tightly to the FRs and trigger cellular uptake *via* endocytosis [143,144]. Recently, FA molecules were functioned as blockers on cationic polyrotaxanes (PR) composed of poly(ethylenimine) (PEI)(600)-grafted α-cyclodextrin rings linearized on polyethylene glycol to form FA-terminated PR-PEI(600) (FPP) for gene delivery (FPP/pDNA). FPP/pDNA showed a lower cytotoxicity, strong specificity to FR, and high efficiency of delivering DNA to target cells *in vitro* and *in vivo* with the reporter genes. Furthermore, the FPP/DNA complex showed an enhanced antitumor effect in the nude mice compared with other delivery systems, such as PEI-25K [145]. Yang et al. developed a FA targeting co-delivery system FA-doxorubicin/Bmi1 siRNA liposome (FA-Dox/siRNA-L). Co-delivery of Bmi1 siRNA and Dox by FA-Dox/siRNA-L exhibited significantly higher efficacy than sole delivery of either Dox or Bmi1 siRNA. Notably, higher accumulation of the siRNA and Dox in tumor cells indicated that FA ligand displayed tumor targeting effect [146]. FA decorated bovine serum albumin (BSA) conjugated carboxymethyl-β-cyclodextrin (CM-β-CD) nanoparticles (FA-BSA-CM-β-CD NPs) were capable of entrapping a hydrophobic Gefitinib. Under the reaction between FA and FRs, Gefitinib loaded FA-BSA-CM-ß-CD NPs induced apoptosis of Hela cells through elevating the expression of caspase-3 and inhibited autophagy through decreasing the expression of LC3 [147]. Interestingly, the application of FA targeting is not confined to solid tumors, but also leukemia cells. FR-β is expressed in approximately 70% of the cases of acute myelogenous leukemia (AML) blast cells. FR-β-targeted liposomal doxorubicin showed higher *in vitro* toxicity than its non-targeted analog in MV4-11 (human acute myelocytic leukemia) and K562 (human erythromyeloblastoid leukemia) cell lines. It also showed stronger inhibition in a colony formation assay with MV4-11, K562 cells and AML patient cells than its non-targeted analog. This suggests the potential clinical application of FA targeting of liposomal nanoparticles in treating AML [148]. However, FA is relatively hydrophobic in nature and strategies to maximize its binding properties on the NPs surface need to be carefully considered and investigated [149]. In addition, an increased surface density of FA ligands on NP surfaces has been shown to give rise to dimers, trimers or tubular quartet self-assembled FA structures which can impede the binding efficiency of FA to its receptors [150].

2.5. Carbohydrates-based targeting molecules

Concerning other molecules that could be used as targeting ligands, carbohydrates have also gained attention due to their ease of production, low molecular weight and high abundance in nature. In addition, sugar chemistry is sufficiently well-known to allow their efficient modification and characterization. Most carbohydrate molecules used for targeting purposes (*e.g.*, galactose, lactose, mannose) bind specifically to asialoglycoprotein receptors (ASGPR), which are membrane lectin receptors commonly found in liver cells [151]. Their multiplicity, high affinity, and effective endocytosis after receptor binding as well as the biocompatibility of carbohydrate ligands render them an interesting ligand for tumor-specific drug delivery [152]. For example, galactose-decorated biodegradable micelles from PEG-b-poly(acryloyl carbonate)-b-PCL and galactose-conjugated PEG-PCL (Gal-PEG-PCL) copolymers were prepared to enhance hepatoma-targeting delivery of PTX. *In vitro* and *in vivo* assays showed that Gal-decorated PTX-loaded micelles retained a high antitumor activity in HepG2 cells, which was much more effective than PTX-loaded cross-linked micelles without Gal ligands [153]. The micelles constructed from PEG-SS-PCL and galactose-PEG-PCL (Gal-PEG-PCL) block copolymers aimed to actively transport Dox into the nuclei of target cancer cells, inducing superb *in vitro* antitumor effects. The results of flow cytometry revealed that cellular Dox level in HepG2 cells treated with Dox-loaded PEG-SS-PCL/Gal micelles was much higher than that with nontargeting PEG-SS-PCL [154]. In another way, mannose and mannan were also used to target mannose receptors on the surface of antigen-presenting cells in order to achieve an improvement of antigen specific response [155]. Silva JM et al. hypothesized that the co-entrapment of melanoma-associated antigens and the Toll-like receptor (TLR) ligands Poly(I:C) and CpG, known to be Th1-immunopotentiators, in mannose-functionalized aliphatic polyester-based nanoparticles could be targeted to mannose receptors on antigen-presenting cells and induce anti-tumor immune responses. The mannose-functionalization of NPs potentiated the

Th1 immune response and the nanoparticulate vaccines indeed decreased the growth rate of murine B16F10 melanoma tumors in therapeutic and prophylatic settings [156].

Polysaccharides, in particular, HA, have gained great interest for targeted drug delivery. HA as natural polyanionic biopolymer has excellent biocompatibility and biodegradability [157]. Given the fact that HA binding receptors, such as CD44 and RHAMM, are overexpressed on the cell surface of several malignant tumors with high metastasis activity, HA has been widely investigated as a targeting constituent of drug carriers for cancer therapy [158,159]. Yoon HY et al. reported tumor-targeting hyaluronic acid nanoparticles (HANPs) as the carrier of the hydrophobic photosensitizer, chlorine6 (Ce6) for simultaneous photodynamic imaging and therapy. The resulting Ce6-HANPs showed stable nano-structure in aqueous condition and rapid uptake into tumor cells. After an intravenous injection into the tumor-bearing mice, Ce6-HANPs could efficiently reach tumor tissue *via* passive targeting mechanism and specifically enter tumor cells through receptor-mediated endocytosis based on the interactions between HA of nanoparticles and CD44 [160,161]. Docetaxel-loaded PLGA-b-HA nanoparticles (DTX/SANPs) also showed enhanced cytotoxicity toward CD44-overexpressing MDA-MB-231 cells. And *in vivo* studies demonstrated that SANPs exhibited enhanced tumor targeting and antitumor activity with lower systemic toxicity [162]. Beyond their targeting abilities, another advantage of HA is the stealth properties they can confer to nanoparticles.

Glycyrrhetinic acid (GA) or glycyrrhizin (GL) is one of the main bioactive compounds extracted from licorice, which could be used to treat hepatitis and hepatotoxicity. On account of their high binding ability to the cellular membrane of hepatocytes, GA or GL has been explored as a specific ligand for hepatoma-targeting chemotherapy [163]. The self-assembly sulfated chitosan nanoparticles functionalized with glycyrrhetinic acid (GA-SCTS) were prepared for liver cancer therapy. Dox-loaded GA-SCTS micelles (Dox/GA-SCTS micelles) had higher affinity for the liver cancer cells (HepG2 cells) than for the normal liver cells (Chang liver cells). There was nearly 2.18-fold improvement in uptake of the Dox/SA-SCTS micelles by HepG2 cells than that by Chang liver cells [164]. O-carboxymethyl chitosan nanoparticles (CMCNP) were modified by glycyrrhizin (GL) as hepatocellular carcinoma (HCC)-targeted delivery vehicles, which could efficiently deliver PTX into HCC. The resultant CMCNP-GL promoted liver cancer SMMC-7721 cell internalization by approximate 10.0-fold as compared to unmodified CMCNP. The *in vivo* tumor inhibition ratio of PTX/CMCNP-GL was 87.5%, showing remarkably higher than that of PTX/CMCNP (64.0%) and PTX injection (34.5%) [165].

2.6. Other emerging targeting small molecules

Small molecules, are usually defined as low molecular weight organic molecules with a molecular weight <500 Da. The use of small molecules to constitute targeted NPs remains a promising targeting strategy due to their small size and ease of handling (less prone to degradation than biomolecular ligands). Some certain unique advantages of small molecule targeting ligands include: (1) the availability of a range of facile coupling chemistries for their conjugation, (2) the possibility to modulate ligand densities and charge on nanoparticle surfaces, since these parameters can affect stability, size, and morphology, as well as targeting efficiency, (3) availability of a wide range of targeting ligands with variable solubilities and functional groups, as facilitated by advances in diversity-oriented synthesis, (4) fewer immunogenic effects *in vivo* (compared to macromolecular ligands) and, (5) reproducible, scalable, and economical manufacturing [44,166].

Small hydrophilic molecules identified from a series of urea-based PSMA inhibitors are capable of targeting the PSMA receptor with affinity and specificity similar to that of antibodies and aptamers [167]. These molecules could enable NPs to be internalized by cells through endocytosis and preferentially accumulate in tumors overexpressing PSMA receptors. Since PSMA is not only highly expressed by prostate cancer but also in the neovasculature of most solid tumors instead of the vasculature of normal tissues, this targeting strategy is quite desirable for therapy and diagnosis applications [168]. Notably, one from this class of molecules, the pseudomimetic dipeptide 2-{[(5-amino-1-carboxypentyl)carbamoyl]aminopentanedioic acid, already used by Sechi et al. for development of (-)-epigallocatechin 3-gallate-loaded PSMA-targeted nanoparticles [169] and by Chandran et al. for preparation of docetaxel-PLA/PCL-based targeted nanoparticles [170], was also used as a targeting ligand in the development of BIND-014 [171]. BIND-014 was the first targeted polymeric nanoparticles for cancer chemotherapy to reach clinical Phase I trials in January 2011. BIND-014 has been demonstrated to be up to 10-fold more effective in delivering docetaxel to tumors with respect to an equivalent dose of free drug in multiple animal models, with no increase in toxicity. The results of a Phase I clinical trial indicate that BIND-014 has a different pharmacologic profile with respect to free docetaxel, including pharmacokinetic properties consistent with a prolonged circulation half-life for BIND-014 and retention of docetaxel in the vascular compartments, with multiple cases of tumor shrinkage at doses up to five times less than the docetaxel dose typically administered. Phase II development of BIND-014 as second-line therapy for patients with non-small-cell lung cancer (NCT01792479) and those with metastatic astrate-resistant prostate cancer (NCT01812746) was started in 2013. Further clinical studies to evaluate BIND-014 in a wide range of solid tumors are currently under consideration [172].

A family of domains referred to as A-domains fulfills all of the criteria for developing a therapeutic protein platform [173]. A-domain proteins, which are 40-amino-acid oligopeptides, bind over 100 different known targets, including small molecules, proteins and viruses [174]. The first A-domain protein was found in the low-density lipoprotein receptor (LDLR) by Tschopp and Mollnes [175]. A new class of targeting molecules, named avimer, has emerged from A-domain proteins. An avimer is a single protein chain containing multiple domains, each of which represents a separate function. All of the avimer clones described show similar expression levels and molecular properties. Promising targets for which avimer binders have been identified include IL-6, cMet, CD28, CD40L and BAFF [176]. Recently, Richard Smith et al. generated avimers using phage-displayed libraries that bound specifically to either FGFR1c or β-Klotho. These domains were then combined into a single polypeptide to generate a bispecific molecule that exhibits potent FGF21-like agonist activity *in vitro* and *in vivo* [177]. Therefore, the avimer platform can generate potent functional molecules for a variety of targets.

Adnectins are a new family of therapeutic oligopeptides based on the 10th fibronectin type III domain, and designed to bind with high affinity and specificity to therapeutically relevant targets. Characterized by thermostability and protease-resistance, each Adnectin typically has three distinct loop structures. A large library of Adnectins has been created by introducing diversity into these loops [178,179]. CT-322, a PEGylated, anti-angiogenic Adnectin, binds the VEGFR-2 extracellular domain with high specificity and affinity to block VEGF-induced VEGFR-2 signaling [180,181]. In Phase I clinical trials, CT-322 had an ~100 h half-life in the bloodstream, was well tolerated, did not give rise to neutralizing antibodies and did not show signs of antibody-mediated clearance. Meantime, CT-322 exhibits promising antitumor activity in patients with advanced solid tumors [182]. Phase II trials are underway to evaluate the efficacy of CT-322, in combination with chemotherapy or with chemotherapy and radiation,

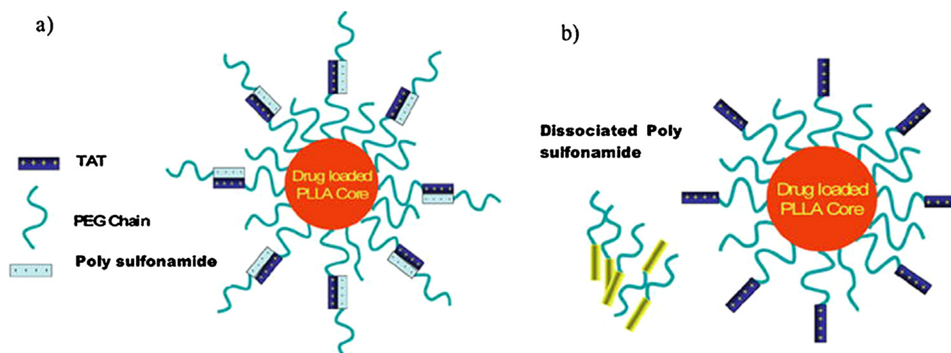

Fig. 4. Schematic model for the proposed drug delivery system: the carrier system consists of two components, a PLLA-b-PEG micelle conjugated to TAT and a pH sensitive diblock polymer PSD-b-PEG. (a) At normal blood pH, the sulfonamide is negatively charged, and when mixed the TATmicelle, shields the TAT by electrostatic interaction. Only PEG is exposed to the outside which could make the carrier long circulating; (b) when the system experiences a decrease in pH (near tumor) sulfonamide looses charge and detaches, thus exposing TAT for interaction with tumor cells.

Source: Adapted from Ref. [188] with kind permission of Y.H. Bae, Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, USA.

against glioblastoma multiforme, non-small cell lung cancer and metastatic colorectal cancer. Conjugation of these molecules on drug-encapsulated NPs may further enhance targeted cancer therapy.

3. TAT exposure by shield/deshielding mechanism

The arrival of targeted NPs directly to tumor cells may be hindered by poor vascularization and permeability within certain tumor regions. This problem is further frustrated by the elevated interstitial pressure of tumors owing to the leaky nature of their blood vessels and their dysfunctional lymphatic vessels which can diminish tumor tissue penetration and retention of NPs [13]. To further retain NPs within tumor tissue and to enhance cellular uptake, cell-penetrating peptides (CPPs) have been used to facilitate translocation of cargoes across the plasma membrane to specific organelles within the cell [183]. Many of these sequences are derived from natural sequences, such as the protein-transduction domains of viruses. The TAT peptide derived from the HIV-1 virus has been popularly used to deliver a variety of NPs into cells [184–186]. In particular, TAT-conjugated fluorescent quantum dots were used as model systems to investigate the cellular tracking of TAT-functionalized NPs [187]. TAT-functionalized NPs efficiently localize in the cells *in vitro*, but they are not suitable for systemic cellular drug delivery. One of the main obstacles that still remain unresolved is the lack of selectivity of TAT in the bloodstream. Once administrated into the bloodstream, the TAT-functionalized NPs would non-selectively distribute in the whole body, and even enter the brain tissues, causing a variety of problems in *in vivo* applications. Thus, these TAT-functionalized NPs must be further designed to overcome the extracellular barriers for *in vivo* applications. An ideal scenario for their *in vivo* applications is to shield non-specific TAT during the blood circulation but expose them once in cancerous tissues or inside intracellular compartments. To this end, Sethuraman and Bae developed an acid-deshielding approach for TAT. The delivery system involves assimilation of two components: (1) chemotherapeutic polymeric micelles – consisting of polyethylene glycol (PEG) outer shell, with TAT attached to the PEG and a hydrophobic core made of poly(L-lactic acid) into which any chemotherapeutic can be incorporated. (2) The TAT shield – an ultra pH sensitive smart block copolymer PSD-b-PEG (Poly sulfonamide). Physical mixing of the two components forms the final carrier. When the PSD-b-PEG polymer is mixed with the TAT micelles, the TAT peptides get shielded by the PSD of the block copolymer (as shown in Fig. 4) [188]. When the shielded and unshielded TAT micelles were investigated for tumor cell internalization at both pH 7.4 and 6.6 by incubating for 1 h, unshielded micelles were internalized into both the cells and its nucleus at pH 7.4 and 6.6. However, the micelle shielded with poly(methacryloyl sulfadimethoxine)-b-PEG had significantly fast uptake of TAT micelles at pH 6.6 compared to pH 7.4, indicating shielding at the normal pH and deshielding at the tumor pH. The confocal microscopy study indicated that the TAT and its cargo not only efficiently entered into the cells but also translocated near the nucleus. Further testing of the delivery system is underway, using chemotherapeutic agents both *in vitro* and *in vivo*, to evaluate its potential to kill tumors. More recently, Lee and Bae fabricated acid-induced pop-up TAT micelles (named as $PHSM^{pop\text{-}upTAT}$) for *in vivo* nuclear targeted delivery of Dox. The $PHSM^{pop\text{-}upTAT}$ is formed by self-assembly of a mixture of two block copolymers of poly(L-lactic acid)(PLA)-b-PEG-b-polyhistidine PHis, TAT and PHis-b-PEG. Then the TAT moieties were anchored on the micelle surface *via* a pH-sensitive PHis with a molecular weight of 2 kDa. At pH 7.4, Phis was water-insoluble and thus the TAT was shielding within the hydrophilic PEG corona shell, preventing the TAT's nonspecific interaction with cells. Once at acidic pH, the Phis became protonated and thus water-soluble, exposing the TAT on the micelle surface for binding. $PHSM^{pop\text{-}upTAT}$ delivered high concentrations of Dox in both the cytosol and the nucleus in various wild and multidrug resistant (MDR) cell lines (3.8–8.8 times lower IC50 than free Dox, depending on cell line). More importantly, when tested with the xenografted tumors of human ovarian tumor drug-resistant A2780/AD, human breast tumor drug-sensitive MCF-7, human lung tumor A549 and human epidermoid tumor KB in a nude mice model, all tumors significantly regressed in size by the Dox-loaded micelles. Results from a series of both *in vitro* and *in vivo* studies strongly support improvement in the treatment of acidic solid tumors, whether they are drug-sensitive or drug-resistant, using the targeting nanotechnology enabled by the $PHSM^{pop\text{-}up\ TAT}$ design [189]. In the future, this design will be extended by using various anticancer drugs and molecular tools for active internalization to achieve a general platform for solid cancer chemotherapy.

4. Tumor pH-active ligand-mediated tumor-targeting delivery

Targeted drug delivery, including ligand-targeted drug delivery, can enhance the therapeutic index by reducing side effects in healthy tissues, while decreasing the overall dose by concentrating the drug in the target tissue. Ligand-targeted drug delivery has been challenged with the help of some receptors overexpressing in the cancerous tissue. However, there has not been a receptor that is expressed on tumor cells exclusively, thus their expression on

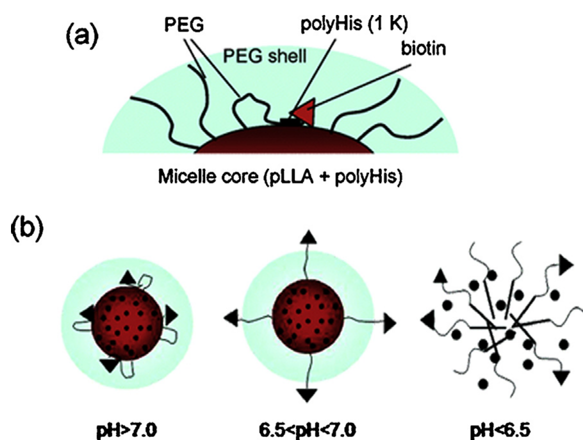

Fig. 5. Schematic diagram showing the central concept of pH-induced vitamin repositioning on the micelle. While above pH 7.0, biotin that is anchored on the micelle core *via* a pH-sensitive molecular chain actuator (polyHis) is shielded by PEG shell of the micelle; biotin is exposed on the micelle surface (6.5 < pH < 7.0) and can interact with cells, which facilitates biotin receptor-mediated endocytosis. When the pH is further lowered (pH < 6.5), the micelle destabilizes, resulting in enhanced drug release and disrupting cell membranes such as endosomal membrane.

Source: Adapted from Ref. [194] with kind permission of Y.H. Bae, Department of Pharmaceutics and Pharmaceutical Chemistry, The University of Utah, Salt Lake City, Utah.

normal cells and tissues may lead to low efficiency and severe toxicity of ligand-targeted drug delivery [190–192]. Characteristics of the tumor microenvironment, such as acidosis, are pervasive in almost all solid tumors and can be easily accessed. It is shown that the different extracellular pH value can be used to activate/inactivate the receptor-mediated endocytosis on tumor/normal cells. Inspired by this, an acid-activated switch was introduced to receptor-mediated transport nanoparticles [193]. Eun Seong Lee et al. developed polymeric micelles with pH-induced ligand repositioning on the micelle surface [194]. As shown in Fig. 5, the core-shell type micelle was constituted from two block copolymer components of poly(L-histidiene) (polyHis)-b-poly(ethylene glycol) (PEG) and poly(L-lactic acid) (pLLA)-b-PEG-b-polyHis-biotin, which was multifunctional; the shorter polyHis block in PLLA-bPEG-b-polyHis-biotin was located at the interface of the hydrophobic core of PLLA and polyHis and the hydrophilic PEG shell, due to the high water solubility of neighboring PEG and biotin. Biotin was selected in this study to demonstrate the proof of concept. The interfacial polyHis caused PEG chain bending and the biotin hiding in the PEG shell, derived from the polyHis-b-PEG block copolymer. As a result, the micelle was stable above pH 7.2 and shields the conjugated biotins. However, with the pH lowered below pH 7.2, the degree of ionization of polyHis increased. The interfacial short polyHis became ionized first and at the critical degree of ionization its hydrophobic interaction with the core phase weakened. As a result, the PEG-b-polyHis-biotin portion expanded, exposing biotin out of the PEG shell. The pH 7.0 seemed the critical pH for this expansion as demonstrated by pH-dependent turbidity of the micelle solution containing avidin, which was a tetrameric protein with four biotin-binding sites. Moreover, when the solution pH was decreased, the relative transparency of the solution was gradually reduced to 10% at a pH range of 6.8–6.0. This was due to the ionization of the polyHis block located in the core and subsequent micelle destabilization by ionized polyHis escaping from the micelle. This process might cause decreased transparency, presumably through certain degree of aggregation of the remaining PLLA-b-PEG block copolymer. In summary, when the environmental pH for the micelle was lowered slightly (Ph ~7.0; tumor acidic pH), biotin was exposed on the micellar surface and could interact with cells, which facilitated biotin receptor-mediated endocytosis. Further lowering the pH (pH < 6.5), the micelle destabilized, resulting in disruption of the endosomal membrane and increased cytosolic drug release. The first functionality was expected to endow tumor pH specificity to nonspecific ligands and the rest may help to treat solid tumors that were hard to treat by conventional chemotherapy (resistant tumors). Recently, DTPA-shielded T7-modified polyethylene glycol (PEG)-conjugated DGL (DGL-PEG-T7-hydrazone-DTPA) was used as the platform for gene delivery (Fig. 6) [195]. DTPA, a hydrophilic molecule, which could prevent the contact of NPs with normal tissue cell membrane in neutral conditions, was chosen as the shielding molecule. Peptide T7 (sequenced by HAIYPRH) was screened by a phage display system on the cells expressing human TfR and has been demonstrated as a potential ligand for targeting delivery of agents to TfR-overexpressed tumor cells [196]. Tumor-targeting peptide T7 was masked by DTPA *via* a pH-sensitive hydrazone linkage. Accordingly, in neutral conditions, targeting ligand T7 was shielded to avoid nonspecific binding. When in the acidic tumor environment, the hydrazone bond could be hydrolyzed to expose ligands for specific tumor targeting. In the UV spectra, the absorbance region of DTPA for DGL-PEG-T7-hydrazone-DTPA disappeared after dialysis at pH 6.5, which indicated a successful deshielding behavior in the acidic tumor environment. In *in vitro* cellular-uptake assays, DGL-PEG-T7-hydrazone-DTPA/pDNA NPs delivered very little pDNA into cells at pH 7.4, however, more pDNA was internalized into cells at pH 6.5. Compared with nonshielding NPs, DGL-PEG-T7/pDNA NPs, which showed efficient gene expression in normal cells and tumor cells at normal conditions, DGL-PEG-T7-hydrazone-DTPA/pDNA NPs could reduce side effects on healthy cells and enhance the tumor-targeting delivery efficiency. This result of the *in vivo* imaging of NPs in tumor-bearing mice showed that TfR-mediated endocytosis for DGL-PEG-T7-hydrazone-DTPA/pDNA NPs may be different from that for DGL-PEG-T7/pDNA NPs. The rate of TfR-mediated endocytosis in tumors for DGL-PEG-T7-hydrazone-DTPA/pDNA was slower than that for DGL-PEG-T7/pDNA NPs. Encouraging was that little signal in normal tissues was shown in mice treated with DGL-PEG-T7-hydrazone-DTPA/pDNA NPs, which suggested that TfR-mediated endocytosis may disappear in normal tissues. Using hydrazone bonding and DTPA to achieve acid-active ligand-mediated tumor-targeting delivery strategies may be broadly applicable for other ligands as well. However, these promising vehicles are only in the basic research phase. In the future, effective acid-active ligand-mediated tumor-targeting NPs with no apparent cytotoxicity and systemic toxicity should be tested in clinical trials. The goal is not only for tumor delivery, but also the avoidance of normal cell uptake.

5. Tumor pH-triggered charge conversion to tumor cell uptake

In contrast to neutral and negatively charged nanoparticles, positively charged particles are the most efficient at cell-membrane penetration and cellular internalization because cationic surfaces will interact with the negatively charged phospholipid head groups, proteins and glycans on the surface of cells [197]. The results of HeLa cell endocytosis of NPs with either a negative or positive charge on their surface suggest that the exposed charge significantly affects not only their internalization capability but also the cellular endocytosis mechanism. Negatively charged NPs illustrate an inferior rate of endocytosis and utilize less the clathrin-mediated endocytosis pathway. On the contrary, the positively charged NPs can be internalized rapidly *via* clathrin-mediated endocytosis. When this pathway is blocked, NPs activate a compensatory endocytosis pathway that leads to even higher accumulation of NPs in cells [198]. Cationic polymers such as poly(ethyleneimine) (PEI) and

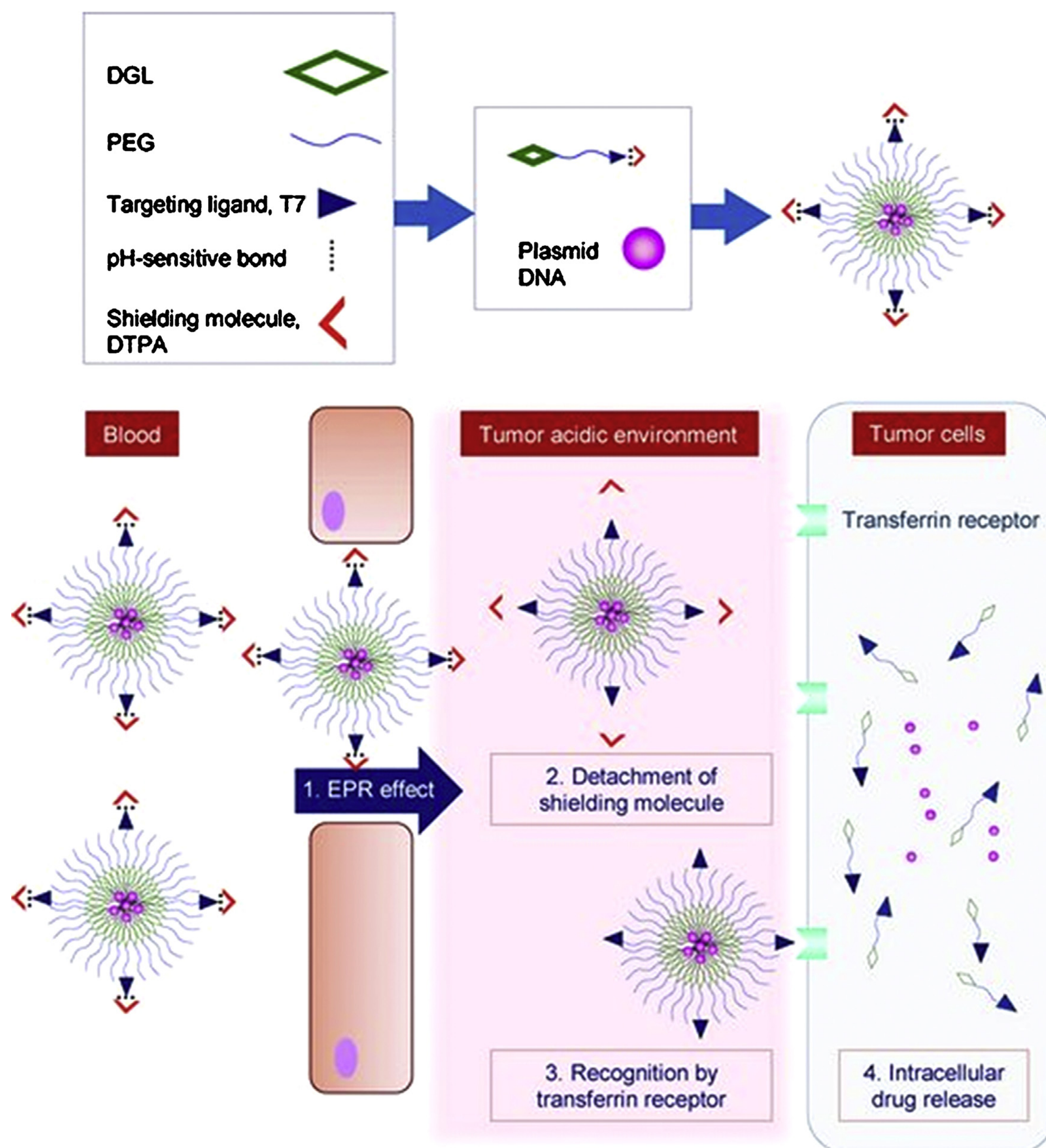

Fig. 6. Acid active receptor-specific peptide ligand-modified tumor-targeting systems for avoiding the uptake by TfR-expressed healthy cells and further amplified tumor accumulation.

Source: Adapted from Ref. [195] with kind permission of C. Jiang, Key Laboratory of Smart Drug Delivery, Ministry of Education & PLA, Department of Pharmaceutics, School of Pharmacy, Fudan University, China.

poly-(L-lysine) (PLL) have been extensively explored for gene delivery. They can carry DNA across the cell membrane, control the molecular motors to actively move along the microtubule network, and finally enter the nucleus [199]. Thus, these cationic polymers might serve as cellular-targeted drug delivery carriers for chemotherapy. However, they contain many primary, secondary amines and thus are highly positively charged at physiological pH, causing strong non-specific cellular uptake in the bloodstream and a variety of problems in *in vivo* applications, including rapid clearance from the blood circulation by cells of the MPS, hemolytic toxicity and hemolysis, formation of holes in the cell membrane and toxicity [200]. Therefore, these positively charged polymers are not favorable as drug carriers for *in vivo* applications.

Recently a "negative-to-positive charge-reversal" approach for cationic polymers was developed to employ their cellular binding properties for *in vivo* tumor cell-targeted drug delivery [201–203]. The cationic charges (from primary and secondary amines) are first masked at the physiological pH so that the carriers can be used for *i.v.* administration, but once inside tumor cells the cationic charges are recovered and thus regain cellular localizing properties. Amides with neighboring carboxylic acid groups show pH-dependent hydrolysis. At neutral pH, the amides are stable and negatively charged because of the β-carboxylic acid groups, while at a low pH, the amides hydrolyze to regenerate the amine groups to carry cationic charges. To demonstrate this concept, a model polymer, polycaprolactone-block-PEI (PCL-PEI) was synthesized [204]. Its PEI block reacted with 1,2-cyclohexanedicarboxylic anhydride to convert the primary and secondary amines into their amides (PCL-PEI/amide). The micelles of PCL-PEI/amid showed a ζ potential of about −20 mV at pH 7.4, even up to 60 h, indicating that they were always negatively charged as a result of the presence of COOH groups. At pH 6, the ζ potential was about +8 mV.

When at pH 5, they immediately became highly positively charged and gradually reached a ζ potential of about + 50 mV in almost 10 h. However, the micelles of PCL-PEI were always positively charged. And *in vitro* cellular uptake experiments, PCL-PEI/amide loaded with Dox entered cells faster at pH 6 than at pH 7.4. Furthermore, the micelles of PCL-PEI/amide loaded with Dox showed no detectable cytotoxicity even at high doses, but significantly increased the antitumor activity of loaded Dox in SKOV-3 cancer cells. Wang and co-workers designed dual pH-sensitive polymer-doxorubicin conjugate as a nanoparticulate drug delivery system to achieve negative-to-positive charge-reversal triggered by the solid tumor extracellular acidity (pH < 7) [205] or lysosomal (pH 4–5) [206]. The nanoparticle was capable of reversing its surface charge from negative to positive at tumor extracellular pH (~6.8) to facilitate cell internalization. Subsequently, the significantly increased acidity in subcellular compartments such as endosomes and lysosomes (pH 5.0) further promoted doxorubicin release from the endocytosed drug carriers *via* pH-triggered hydrolysis of hydrazone bonds linking Dox to the carrier. These dual pH-sensitive nanoparticles have demonstrated enhanced cytotoxicity in drug-resistant cancer stem cells [207]. In addition, Yang et al. reported reversibly stealth cationic micelles based on a novel pH responsible benzoic imine linker in the micelle-forming amphiphilic copolymer for tumor-specific uptake and intracellular drug release [208,209]. The benzoic-imine linker is stable at physiological pH, partially hydrolyzes at the extracellular pH of the solid tumor, and completely hydrolyzes at the endosomal pH. Meanwhile, at physiological pH, the micellar surface was shielded by the PEG corona, leading to lower cytotoxicity and less hemolysis, whereas in a mild acidic condition like in solid tumors, the deshielding of the PEG chains exposed the positive charge on the micellar surface due to the generation of amino groups from the cleavage of the imine bond. The ionization on the surface facilitated the tumor cell uptake of the micelles through the electrostatic interaction between the micelle and the cell membrane. Subsequently, at the endosomal pH, with more complete cleavage of the polymer the micellar structure dissociated, and the system became quite membrane-disruptive, indicating an enhanced intracellular delivery capability *via* the endosomal pathway. The amphiphilic "stealth" polycation is a highly appealing alternative to cell-specific "active" targeting method.

6. Potential of nanoparticles to overcome drug resistance

The delivery of drugs through targeted nanocarriers provides an alternative route to transport drugs into cells. This approach may allow targeted carriers to bypass the activity of integral membrane proteins, known as MDR transporters, which transport a variety of anticancer drugs out of the cancer cell and produce resistance against chemotherapy [210]. The molecular basis of cancer drug resistance is complex and has been correlated to elevated levels of enzymes that can neutralize chemotherapeutic drugs. More often, however, it is due to the overexpression of MDR transporters that actively pump chemotherapeutic drugs out of the cell and reduce the intracellular drug doses below lethal threshold levels. Since not all cancer cells express the MDR transporters, only drug-sensitive cells that do not or only mildly express MDR transporters were killed by chemotherapy, while leaving alone a small population of drug resistant cells that highly express MDR transporters. With tumor recurrence, chemotherapy may fail because residual drug-resistant cells dominate the tumor population [55]. Among the MDR transporters, the most widely investigated proteins are: P-glycoprotein (also referred to as MDR1 or ABCB1); the multidrug resistance associated proteins (MRPs), of which the most studied is the MRP1 (or ABCC1); and the breast cancer resistance protein

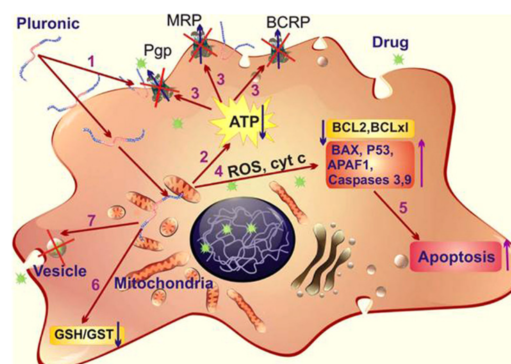

Fig. 7. Multiple effects of Pluronic block copolymers in MDR cells: (1) incorporation of Pluronic molecules into membranes and decrease of the membrane microviscosity; (2) induction of ATP depletion; (3) inhibition of drug efflux transporters; (4) release of cytochrome C from mitochondria and increase in ROS levels in cytoplasm; (5) increase of pro-apoptotic signaling and decrease of anti-apoptotic defense in MDR cells; (6) inhibition of the glutathione/glutathione S-transferase detoxification system; and (7) abolishment of drug sequestration within cytoplasmic vesicles.
Source: Adapted from Ref. [213] with kind permission of E.V. Batrakova, Center for Drug Delivery and Nanomedicine, Department of Pharmaceutical Sciences, University of Nebraska Medical Center, USA.

(ABCG2). These proteins have different structures, but they form a unique defense against chemotherapeutics and numerous endo- and exotoxins [211].

Pluronic block copolymers have sparked a considerable interest as potential drug carriers, as they can cause exceeding sensitization of MDR tumors to anticancer agents [212]. The biological activity of these amphiphilic block copolymers is based on their ability to incorporate into membranes followed by subsequent translocation into the cells and alteration of various cellular functional functions, such as mitochondrial respiration, ATP synthesis, drug efflux transport, apoptotic signal transduction, and gene expression (Fig. 7) [213]. According to this multiple action, pluronics can also enrich drug transport across the blood brain and intestinal barriers, and transcriptional activation of gene expression. As an example, Dox incorporated in mixed micelles of pluronic block copolymers, termed as SP1049C, had the unique ability to chemosensitize MDR tumors by inhibiting the P-gp drug efflux system, and enhancing the pro-apoptotic signaling in cancer cells. Efficacy of SP1049C was confirmed in *in vivo* experiments in both sensitive and resistant tumor models, including P388 and P388/ADR murine leukemia, Sp2/0 and Sp2/0Dnr murine myeloma, 3LL-M27 Lewis lung carcinoma, MCF-7 and MCF-7/ADR human breast carcinomas, and KBv human oral epidermoid carcinoma. And SP1049C has shown a very interesting activity as monotherapy in patients with advanced esophageal carcinoma [213–215].

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.[216] In order to overcome multidrug resistance in solid tumors, Dox loaded pH-sensitive micelles of which surface was decorated with folate (PHSM/f) were evaluated both *in vitro* and *in vivo* experiments. The PHSM/f exhibited greater inhibitory activity against drug-resistant MCF-7 cells and/or xenografts than their nontargeted free drug counterparts. In the drug-resistant MCF-7 xenograft model, the accumulated Dox level of PHSM/f in solid tumors was 20 times higher than that of free Dox group, and 3 times higher than that of PHSM group [217]. Transferrin-conjugated paclitaxel nanoparticles and transferrin-ligated liposomes containing oxaliplatin are other examples of ligand-targeted NPs, which exhibited greater antitumor activity than the respective free drugs in drug-resistant mouse models [218,219]. Combination treatments with targeted

nanocarriers for selective delivery of drugs and MDR pump inhibitors also likely address some of the problems posed by resistant tumors. Vincristine-loaded liposomes conjugated to MRK-16 (a MAb against P-gp) have induced an enhancement of the drug cytotoxicity against resistant human myelogenous leukemia cell lines, compared to conventional vincristine-loaded liposomes. This enhanced efficacy was attributed to the inhibition of P-gp mediated efflux of vincristine by MRK-16 [220].

Drug resistance continues to be a primary hindrance for the efficiency of cancer chemotherapy. Novel nanoparticles are rapidly evolving and indeed improve anticancer efficacy on multidrug-resistant tumors *in vitro* and *in vivo*. However, the mechanisms to overcome MDR using these nanoparticles are complicated and not fully understood. More detailed studies on mechanisms would help direct the application of current delivery systems or lead to the discovery of alternative novel delivery systems.

7. Conclusion

The past decade has witnessed an explosive development of drug-loaded polymeric nanoparticles for enhanced internalization in cancer therapy. It should be noted, however, that many of these novel concepts have to be proved in different animal models and eventually in human patients. Ultimately the question remains as to whether these multifunctional targeted NPs demonstrate marked improvement in clinical outcomes, which need to be demonstrated through well executed larger clinical trials. Beyond the regulatory requirements of demonstrating safety, efficacy, quality and cost-effectiveness, further challenges of each multifunctional targeted NPs technology need to be investigated on a case-by-case basis and these challenges must be met in order to harness their tremendous potential as a new class of targeted therapeutics.

References

[1] S.M. Moghimi, A.C. Hunter, J.C. Murray, Long-circulating and target-specific nanoparticles: theory to practice, Pharmacol. Rev. 53 (2001) 283–318.
[2] M.E. Davis, Z.G. Chen, D.M. Shin, Nanoparticle therapeutics: an emerging treatment modality for cancer, Nat. Rev. Drug Discov. 7 (2008) 771–782.
[3] T. Lammers, W.E. Hennink, G. Storm, Tumour-targeted nanomedicines: principles and practice, Br. J. Cancer 99 (2008) 392–397.
[4] R.A. Petros, J.M. DeSimone, Strategies in the design of nanoparticles for therapeutic applications, Nat. Rev. Drug Discov. 9 (2010) 615–627.
[5] C.M. Paleos, D. Tsiourvas, Z. Sideratou, L.A. Tziveleka, Drug delivery using multifunctional dendrimers and hyperbranched polymers, Expert Opin. Drug Deliv. 7 (2010) 1387–1398.
[6] F. Danhier, O. Feron, V. Preat, To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery, J. Control. Release 148 (2010) 135–146.
[7] S. Biswas, V.P. Torchilin, Nanopreparations for organelle-specific delivery in cancer, Adv. Drug Deliv. Rev. 66 (2014) 26–41.
[8] O.C. Farokhzad, R. Langer, Impact of nanotechnology on drug delivery, ACS Nano 3 (2009) 16–20.
[9] A. Gabizon, R. Catane, B. Uziely, B. Kaufman, T. Safra, R. Cohen, F. Martin, A. Huang, Y. Barenholz, Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes, Cancer Res. 54 (1994) 987–992.
[10] T.Y. Kim, D.W. Kim, J.Y. Chung, S.G. Shin, S.C. Kim, D.S. Heo, N.K. Kim, Y.J. Bang, Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies, Clin. Cancer Res. 10 (2004) 3708–3716.
[11] S.R. Park, D.Y. Oh, D.W. Kim, T.Y. Kim, D.S. Heo, Y.J. Bang, N.K. Kim, W.K. Kang, H.T. Kim, S.A. Im, J.H. Suh, H.K. Kim, H.K. Kim, A multi-center, late phase II clinical trial of Genexol (paclitaxel) and cisplatin for patients with advanced gastric cancer, Oncol. Rep. 12 (2004) 1059–1064.
[12] Y. Matsumura, T. Hamaguchi, T. Ura, K. Muro, Y. Yamada, Y. Shimada, K. Shirao, T. Okusaka, H. Ueno, M. Ikeda, N. Watanabe, Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin, Br. J. Cancer 91 (2004) 1775–1781.
[13] R.K. Jain, T. Stylianopoulos, Delivering nanomedicine to solid tumors, Nat. Rev. Clin. Oncol. 7 (2010) 653–664.
[14] T. Lammers, F. Kiessling, W.E. Hennink, G. Storm, Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress, J. Control. Release 161 (2012) 175–187.
[15] V. Torchilin, Tumor delivery of macromolecular drugs based on the EPR effect, Adv. Drug Deliv. Rev. 63 (2011) 131–135.
[16] J. Gong, M. Chen, Y. Zheng, S. Wang, Y. Wang, Polymeric micelles drug delivery system in oncology, J. Controll. Release 159 (2012) 312–323.
[17] H. Hatakeyama, H. Akita, H. Harashima, A multifunctional envelope type nano device (MEND) for gene delivery to tumours based on the EPR effect: a strategy for overcoming the PEG dilemma, Adv. Drug Deliv. Rev. 63 (2011) 152–160.
[18] B. Romberg, W.E. Hennink, G. Storm, Sheddable coatings for long-circulating nanoparticles, Pharm. Res. 25 (2008) 55–71.
[19] M. Ferrari, Cancer nanotechnology: opportunities and challenges, Nat. Rev. Cancer 5 (2005) 161–171.
[20] M.M. Gottesman, T. Fojo, S.E. Bates, Multidrug resistance in cancer: role of ATP-dependent transporters, Nat. Rev. Cancer 2 (2002) 48–58.
[21] D. Peer, R. Margalit, Fluoxetine and reversal of multidrug resistance, Cancer Lett. 237 (2006) 180–187.
[22] K.C. Gatter, G. Brown, I.S. Trowbridge, R.E. Woolston, D.Y. Mason, Transferrin receptors in human tissues: their distribution and possible clinical relevance, J. Clin. Pathol. 36 (1983) 539–545.
[23] D.B. Kirpotin, D.C. Drummond, Y. Shao, M.R. Shalaby, K. Hong, U.B. Nielsen, J.D. Marks, C.C. Benz, J.W. Park, Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models, Cancer Res. 66 (2006) 6732–6740.
[24] D.W. Bartlett, H. Su, I.J. Hildebrandt, W.A. Weber, M.E. Davis, Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 15549–15554.
[25] R. Vivek, R. Thangam, V. NipunBabu, C. Rejeeth, S. Sivasubramanian, P. Gunasekaran, K. Muthuchelian, S. Kannan, Multifunctional HER2-antibody conjugated polymeric nanocarrier-based drug delivery system for multi-drug-resistant breast cancer therapy, ACS Appl. Mater. Interfaces 6 (2014) 6469–6480.
[26] H.W. Yang, Y.J. Lu, K.J. Lin, S.C. Hsu, C.Y. Huang, S.H. She, H.L. Liu, C.W. Lin, M.C. Xiao, S.P. Wey, P.Y. Chen, T.C. Yen, K.C. Wei, C.C. Ma, EGRF conjugated PEGylated nanographene oxide for targeted chemotherapy and photothermal therapy, Biomaterials 34 (2013) 7204–7214.
[27] J. Ahn, Y. Miura, N. Yamada, T. Chida, X. Liu, A. Kim, R. Sato, R. Tsumura, Y. Koga, M. Yasunaga, N. Nishiyama, Y. Matsumura, H. Cabral, K. Kataoka, Antibody fragment-conjugated polymeric micelles incorporating platinum drugs for targeted therapy of pancreatic cancer, Biomaterials 39 (2015) 23–30.
[28] C. Jin, N. Qian, W. Zhao, W. Yang, L. Bai, H. Wu, M. Wang, W. Song, K. Dou, Improved therapeutic effect of DOX-PLGA-PEG micelles decorated with bivalent fragment HAb18 F(ab′)(2) for hepatocellular carcinoma, Biomacromolecules 11 (2010) 2422–2431.
[29] Y. Nishimura, H. Mieda, J. Ishii, C. Ogino, T. Fujiwara, A. Kondo, Targeting cancer cell-specific RNA interference by siRNA delivery using a complex carrier of affibody-displaying bio-nanocapsules and liposomes, J. Nanobiotechnol. 11 (2013) 19.
[30] M. Talelli, S. Oliveira, C.J. Rijcken, E.H. Pieters, T. Etrych, K. Ulbrich, R.C. van Nostrum, G. Storm, W.E. Hennink, T. Lammers, Intrinsically active nanobody-modified polymeric micelles for tumor-targeted combination therapy, Biomaterials 34 (2013) 1255–1260.
[31] W. Zhou, Y. Zhou, J. Wu, Z. Liu, H. Zhao, J. Liu, J. Ding, Aptamer-nanoparticle bioconjugates enhance intracellular delivery of vinorelbine to breast cancer cells, J. Drug Target. 22 (2014) 57–66.
[32] S.E. Baek, K.H. Lee, Y.S. Park, D.K. Oh, S. Oh, K.S. Kim, D.E. Kim, RNA aptamer-conjugated liposome as an efficient anticancer drug delivery vehicle targeting cancer cells in vivo, J. Control. Release 196C (2014) 234–242.
[33] X. Huang, S. Schwind, B. Yu, R. Santhanam, H. Wang, P. Hoellerbauer, A. Mims, R. Klisovic, A.R. Walker, K.K. Chan, W. Blum, D. Perrotti, J.C. Byrd, C.D. Bloomfield, M.A. Caligiuri, R.J. Lee, R. Garzon, N. Muthusamy, L.J. Lee, G. Marcucci, Targeted delivery of microRNA-29b by transferrin-conjugated anionic lipopolyplex nanoparticles: a novel therapeutic strategy in acute myeloid leukemia, Clin. Cancer Res. 19 (2013) 2355–2367.
[34] Z. Su, L. Xing, Y. Chen, Y. Xu, F. Yang, C. Zhang, Q. Ping, Y. Xiao, Lactoferrin-modified poly(ethylene glycol)-grafted BSA nanoparticles as a dual-targeting carrier for treating brain gliomas, Mol. Pharm. 11 (2014) 1823–1834.
[35] X. He, C.S. Alves, N. Oliveira, J. Rodrigues, J. Zhu, I. Banyai, H. Tomas, X. Shi, RGD peptide-modified multifunctional dendrimer platform for drug encapsulation and targeted inhibition of cancer cells, Colloids Surf. B: Biointerfaces 125C (2014) 82–89.
[36] Y. Miura, T. Takenaka, K. Toh, S. Wu, H. Nishihara, M.R. Kano, Y. Ino, T. Nomoto, Y. Matsumoto, H. Koyama, H. Cabral, N. Nishiyama, K. Kataoka, Cyclic RGD-linked polymeric micelles for targeted delivery of platinum anticancer drugs to glioblastoma through the blood-brain tumor barrier, ACS Nano 7 (2013) 8583–8592.
[37] J. Shen, Q. Meng, H. Sui, Q. Yin, Z. Zhang, H. Yu, Y. Li, iRGD conjugated TPGS mediates codelivery of paclitaxel and survivin shRNA for the reversal of lung cancer resistance, Mol. Pharm. 11 (2014) 2579–2591.
[38] Z. Yan, F. Wang, Z. Wen, C. Zhan, L. Feng, Y. Liu, X. Wei, C. Xie, W. Lu, LyP-1-conjugated PEGylated liposomes: a carrier system for targeted therapy of lymphatic metastatic tumor, J. Control. Release 157 (2012) 118–125.

[39] J.J. Pillai, A.K. Thulasidasan, R.J. Anto, D.N. Chithralekha, A. Narayanan, G.S. Kumar, Folic acid conjugated cross-linked acrylic polymer (FA-CLAP) hydrogel for site specific delivery of hydrophobic drugs to cancer cells, J Nanobiotechnol. 12 (2014) 25.
[40] Y.C. Chen, C.F. Chiang, L.F. Chen, P.C. Liang, W.Y. Hsieh, W.L. Lin, Polymersomes conjugated with des-octanoyl ghrelin and folate as a BBB-penetrating cancer cell-targeting delivery system, Biomaterials 35 (2014) 4066–4081.
[41] X. Wang, H. Sun, F. Meng, R. Cheng, C. Deng, Z. Zhong, Galactose-decorated reduction-sensitive degradable chimaeric polymersomes as a multifunctional nanocarrier to efficiently chaperone apoptotic proteins into hepatoma cells, Biomacromolecules 14 (2013) 2873–2882.
[42] H.Y. Yoon, H. Koo, K.Y. Choi, I. Chan Kwon, K. Choi, J.H. Park, K. Kim, Photo-crosslinked hyaluronic acid nanoparticles with improved stability for in vivo tumor-targeted drug delivery, Biomaterials 34 (2013) 5273–5280.
[43] J. Hrkach, D. Von Hoff, M. Mukkaram Ali, E. Andrianova, J. Auer, T. Campbell, D. De Witt, M. Figa, M. Figueiredo, A. Horhota, S. Low, K. McDonnell, E. Peeke, B. Retnarajan, A. Sabnis, E. Schnipper, J.J. Song, Y.H. Song, J. Summa, D. Tompsett, G. Troiano, T. Van Geen Hoven, J. Wright, P. LoRusso, P.W. Kantoff, N.H. Bander, C. Sweeney, O.C. Farokhzad, R. Langer, S. Zale, Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile, Sci. Transl. Med. 4 (2012), 128ra139.
[44] N. Kamaly, Z. Xiao, P.M. Valencia, A.F. Radovic-Moreno, O.C. Farokhzad, Targeted polymeric therapeutic nanoparticles: design, development and clinical translation, Chem. Soc. Rev. 41 (2012) 2971–3010.
[45] J.-M. Montenegro, V. Grazu, A. Sukhanova, S. Agarwal, J.M. de la Fuente, I. Nabiev, A. Greiner, W.J. Parak, Controlled antibody/(bio-) conjugation of inorganic nanoparticles for targeted delivery, Adv. Drug Del. Rev. 65 (2013) 677–688.
[46] C. Kim, J.F. Galloway, K.H. Lee, P.C. Searson, Universal antibody conjugation to nanoparticles using the Fcgamma receptor I (FcgammaRI): quantitative profiling of membrane biomarkers, Bioconjug. Chem. 25 (2014) 1893–1901.
[47] S. Maya, B. Sarmento, V.K. Lakshmanan, D. Menon, R. Jayakumar, Actively targeted cetuximab conjugated gamma-poly(glutamic acid)-docetaxel nanomedicines for epidermal growth factor receptor over expressing colon cancer cells, J. Biomed. Nanotechnol. 10 (2014) 1416–1428.
[48] N. Karra, T. Nassar, A.N. Ripin, O. Schwob, J. Borlak, S. Benita, Antibody conjugated PLGA nanoparticles for targeted delivery of paclitaxel palmitate: efficacy and biofate in a lung cancer mouse model, Small 9 (2013) 4221–4236.
[49] L. Nobs, F. Buchegger, R. Gurny, E. Allemann, Biodegradable nanoparticles for direct or two-step tumor immunotargeting, Bioconjug. Chem. 17 (2006) 139–145.
[50] L. Nobs, F. Buchegger, R. Gurny, E. Allemann, Poly(lactic acid) nanoparticles labeled with biologically active Neutravidin for active targeting, Eur. J. Pharm. Biopharm. 58 (2004) 483–490.
[51] H. Han, M.E. Davis, Single-antibody, targeted nanoparticle delivery of camptothecin, Mol, Pharm. 10 (2013) 2558–2567.
[52] H. Han, M.E. Davis, Targeted nanoparticles assembled via complexation of boronic-acid-containing targeting moieties to diol-containing polymers, Bioconjug. Chem. 24 (2013) 669–677.
[53] H.W. Kao, Y.Y. Lin, C.C. Chen, K.H. Chi, D.C. Tien, C.C. Hsia, W.J. Lin, F.D. Chen, M.H. Lin, H.E. Wang, Biological characterization of cetuximab-conjugated gold nanoparticles in a tumor animal model, Nanotechnology 25 (2014) 295102.
[54] F.R. Brennan, L. Shaw, M.G. Wing, C. Robinson, Preclinical safety testing of biotechnology-derived pharmaceuticals, Mol. Biotechnol. 27 (2004) 59–74.
[55] D. Peer, J.M. Karp, S. Hong, O.C. Farokhzad, R. Margalit, R. Langer, Nanocarriers as an emerging platform for cancer therapy, Nat. Nanotechnol. 2 (2007) 751–760.
[56] M.M. Cardoso, I.N. Peca, A.C. Roque, Antibody-conjugated nanoparticles for therapeutic applications, Curr. Med. Chem. 19 (2012) 3103–3127.
[57] Y. Matsumura, M. Gotoh, K. Muro, Y. Yamada, K. Shirao, Y. Shimada, M. Okuwa, S. Matsumoto, Y. Miyata, H. Ohkura, K. Chin, S. Baba, T. Yamao, A. Kannami, Y. Takamatsu, K. Ito, K. Takahashi, Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer, Ann. Oncol. 15 (2004) 517–525.
[58] T. Hamaguchi, Y. Matsumura, Y. Nakanishi, K. Muro, Y. Yamada, Y. Shimada, K. Shirao, H. Niki, S. Hosokawa, T. Tagawa, T. Kakizoe, Antitumor effect of MCC-465, pegylated liposomal doxorubicin tagged with newly developed monoclonal antibody GAH, in colorectal cancer xenografts, Cancer Sci. 95 (2004) 608–613.
[59] N. Senzer, J. Nemunaitis, D. Nemunaitis, C. Bedell, G. Edelman, M. Barve, R. Nunan, K.F. Pirollo, A. Rait, E.H. Chang, Phase I study of a systemically delivered p53 nanoparticle in advanced solid tumors, Mol. Ther. 21 (2013) 1096–1103.
[60] C. Qian, Y. Wang, Y. Chen, L. Zeng, Q. Zhang, X. Shuai, K. Huang, Suppression of pancreatic tumor growth by targeted arsenic delivery with anti-CD44v6 single chain antibody conjugated nanoparticles, Biomaterials 34 (2013) 6175–6184.
[61] A.T. Orlova, V. Pehrson, R. Lindborg, M. Tran, T. Sandström, M. Nilsson, F. Wennborg, A. Abrahmsen, L.J. Feldwisch, Cancer Res. 67, 2178–2186 CrossRef, PubMed CAS, Web of Science® Times Cited, 2007, pp. 63.
[62] M. Lindborg, A. Dubnovitsky, K. Olesen, T. Bjorkman, L. Abrahmsen, J. Feldwisch, T. Hard, High-affinity binding to staphylococcal protein A by an engineered dimeric Affibody molecule, Protein Eng. Des. Sel. 26 (2013) 635–644.
[63] V. Tolmachev, T.A. Tran, D. Rosik, A. Sjöberg, L. Abrahmsén, A. Orlova, Tumor targeting using affibody molecules: interplay of affinity, target expression level, and binding site composition, J. Nucl. Med. 53 (2012) 953–960.
[64] M. Satpathy, L. Wang, R. Zielinski, W. Qian, M. Lipowska, J. Capala, G.Y. Lee, H. Xu, Y.A. Wang, H. Mao, L. Yang, Active targeting using HER-2-affibody-conjugated nanoparticles enabled sensitive and specific imaging of orthotopic HER-2 positive ovarian tumors, Small 10 (2014) 544–555.
[65] H. Yang, W. Cai, L. Xu, X. Lv, Y. Qiao, P. Li, H. Wu, Y. Yang, L. Zhang, Y. Duan, Nanobubble-affibody: novel ultrasound contrast agents for targeted molecular ultrasound imaging of tumor, Biomaterials 37 (2015) 279–288.
[66] J. Beuttler, M. Rothdiener, D. Müller, F.Y. Frejd, R.E. Kontermann, Targeting of epidermal growth factor receptor (EGFR)-expressing tumor cells with sterically stabilized affibody liposomes (SAL), Bioconjug. Chem. 20 (2009) 1201–1208.
[67] M. Yang, K. Cheng, S. Qi, H. Liu, Y. Jiang, H. Jiang, J. Li, K. Chen, H. Zhang, Z. Cheng, Affibody modified and radiolabeled gold-iron oxide hetero-nanostructures for tumor PET, optical and MR imaging, Biomaterials 34 (2013) 2796–2806.
[68] R. Heukers, I. Altintas, S. Raghoenath, E. De Zan, R. Pepermans, R.C. Roovers, R. Haselberg, W.E. Hennink, R.M. Schiffelers, R.J. Kok, P.M. van Bergen en Henegouwen, Targeting hepatocyte growth factor receptor (Met) positive tumor cells using internalizing nanobody-decorated albumin nanoparticles, Biomaterials 35 (2014) 601–610.
[69] A. Mujić-Delić, R.H. de Wit, F. Verkaar, M.J. Smit, GPCR-targeting nanobodies: attractive research tools, diagnostics, and therapeutics, Trends Pharmacol Sci. (2014).
[70] S. Oliveira, R. Heukers, J. Sornkom, R.J. Kok, P.M. van Bergen en Henegouwen, Targeting tumors with nanobodies for cancer imaging and therapy, J. Control. Release 172 (2013) 607–617.
[71] P.B. van Driel, J.R. van der Vorst, F.P. Verbeek, S. Oliveira, T.J. Snoeks, S. Keereweer, B. Chan, M.C. Boonstra, J.V. Frangioni, P.M. van Bergen en Henegouwen, A.L. Vahrmeijer, C.W. Lowik, Intraoperative fluorescence delineation of head and neck cancer with a fluorescent anti-epidermal growth factor receptor nanobody, Int. J. Cancer 134 (2014) 2663–2673.
[72] K. Van Impe, J. Bethuyne, S. Cool, F. Impens, D. Ruano-Gallego, O. De Wever, B. Vanloo, M. Van Troys, K. Lambein, C. Boucherie, E. Martens, O. Zwaenepoel, G. Hassanzadeh-Ghassabeh, J. Vandekerckhove, K. Gevaert, L.A. Fernandez, N.N. Sanders, J. Gettemans, A nanobody targeting the F-actin capping protein CapG restrains breast cancer metastasis, Breast Cancer Res. 15 (2013) R116.
[73] I. Altintas, R. Heukers, R. van der Meel, M. Lacombe, M. Amidi, P.M. van Bergen en Henegouwen, W.E. Hennink, R.M. Schiffelers, R.J. Kok, Nanobody-albumin nanoparticles (NANAPs) for the delivery of a multikinase inhibitor 17864 to EGFR overexpressing tumor cells, J. Control. Release 165 (2013) 110–118.
[74] M. Cho, S.S. Oh, J. Nie, R. Stewart, M.J. Radeke, M. Eisenstein, P.J. Coffey, J.A. Thomson, H.T. Soh, Array-based discovery of aptamer pairs, Anal. Chem. (2014).
[75] R. Stoltenburg, C. Reinemann, B. Strehlitz, SELEX—a (r) evolutionary method to generate high-affinity nucleic acid ligands, Biomol. Eng. 24 (2007) 381–403.
[76] C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase, Sci 249 (1990) 505–510.
[77] A.D. Ellington, J.W. Szostak, In vitro selection of RNA molecules that bind specific ligands, Nature 346 (1990) 818–822.
[78] J. Zhu, H. Huang, S. Dong, L. Ge, Y. Zhang, Progress in aptamer-mediated drug delivery vehicles for cancer targeting and its implications in addressing chemotherapeutic challenges, Theranostics 4 (2014) 931–944.
[79] D. Musumeci, D. Montesarchio, Polyvalent nucleic acid aptamers and modulation of their activity: a focus on the thrombin binding aptamer, Pharmacol. Ther. 136 (2012) 202–215.
[80] H. Zhu, J. Li, X.B. Zhang, M. Ye, W. Tan, Nucleic acid aptamer-mediated drug delivery for targeted cancer therapy, ChemMedChem (2014).
[81] F.X. Gu, R. Karnik, A.Z. Wang, F. Alexis, E. Levy-Nissenbaum, S. Hong, R.S. Langer, O.C. Farokhzad, Targeted nanoparticles for cancer therapy, Nano Today 2 (2007) 14–21.
[82] J.H. Lee, M.V. Yigit, D. Mazumdar, Y. Lu, Molecular diagnostic and drug delivery agents based on aptamer-nanomaterial conjugates, Adv. Drug Del. Rev. 62 (2010) 592–605.
[83] J.F. Lee, J.R. Hesselberth, L.A. Meyers, A.D. Ellington, Aptamer database, Nucleic Acids Res. 32 (2004) D95–D100.
[84] Y.H. Lao, K.K. Phua, K.W. Leong, Aptamer nanomedicine for cancer therapeutics: barriers and potential for translation, ACS Nano 9 (2015) 2235–2254.
[85] C.E. Tucker, L.S. Chen, M.B. Judkins, J.A. Farmer, S.C. Gill, D.W. Drolet, Detection and plasma pharmacokinetics of an anti-vascular endothelial growth factor oligonucleotide-aptamer (NX1838) in rhesus monkeys, J. Chromatogr. B: Biomed. Sci. Appl. 732 (1999) 203–212.
[86] E.S. Gragoudas, A.P. Adamis, E.T. Cunningham Jr., M. Feinsod, D.R. Guyer, Pegaptanib for neovascular age-related macular degeneration, N. Engl. J. Med. 351 (2004) 2805–2816.

[87] X. Li, Q. Zhao, L. Qiu, Smart ligand: aptamer-mediated targeted delivery of chemotherapeutic drugs and siRNA for cancer therapy, J. Control. Release 171 (2013) 152–162.
[88] V. de Franciscis, A theranostic "SMART" aptamer for targeted therapy of prostate cancer, Mol. Ther. 22 (2014) 1886–1888.
[89] L. Tang, R. Tong, V.J. Coyle, Q. Yin, H. Pondenis, L.B. Borst, J. Cheng, T.M. Fan, Targeting tumor vasculature with aptamer-functionalized Doxorubicin-polylactide nanoconjugates for enhanced cancer therapy, ACS Nano 9 (2015) 5072–5081.
[90] L. Li, J. Hou, X. Liu, Y. Guo, Y. Wu, L. Zhang, Z. Yang, Nucleolin-targeting liposomes guided by aptamer AS1411 for the delivery of siRNA for the treatment of malignant melanomas, Biomaterials 35 (2014) 3840–3850.
[91] B.T. Ristau, D.S. O'Keefe, D.J. Bacich, The prostate-specific membrane antigen: lessons and current clinical implications from 20 years of research, Urol. Oncol. 32 (2014) 272–279.
[92] S. Dhar, N. Kolishetti, S.J. Lippard, O.C. Farokhzad, Targeted delivery of a cisplatin prodrug for safer and more effective prostate cancer therapy in vivo, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 1850–1855.
[93] N. Kolishetti, S. Dhar, P.M. Valencia, L.Q. Lin, R. Karnik, S.J. Lippard, R. Langer, O.C. Farokhzad, Engineering of self-assembled nanoparticle platform for precisely controlled combination drug therapy, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 17939–17944.
[94] N. Zhao, J. You, Z. Zeng, C. Li, Y. Zu, An ultra pH-sensitive and aptamer-equipped nanoscale drug-delivery system for selective killing of tumor cells, Small 9 (2013) 3477–3484.
[95] L. Li, D. Xiang, S. Shigdar, W. Yang, Q. Li, J. Lin, K. Liu, W. Duan, Epithelial cell adhesion molecule aptamer functionalized PLGA-lecithin-curcumin-PEG nanoparticles for targeted drug delivery to human colorectal adenocarcinoma cells, Int. J. Nanomed. 9 (2014) 1083–1096.
[96] H. Gao, J. Qian, Z. Yang, Z. Pang, Z. Xi, S. Cao, Y. Wang, S. Pan, S. Zhang, W. Wang, X. Jiang, Q. Zhang, Whole-cell SELEX aptamer-functionalised poly(ethyleneglycol)-poly(epsilon-caprolactone) nanoparticles for enhanced targeted glioblastoma therapy, Biomaterials 33 (2012) 6264–6272.
[97] C.H. Choi, C.A. Alabi, P. Webster, M.E. Davis, Mechanism of active targeting in solid tumors with transferrin-containing gold nanoparticles, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 1235–1240.
[98] W.M. Pardridge, Blood-brain barrier drug targeting enables neuroprotection in brain ischemia following delayed intravenous administration of neurotrophins, Adv. Exp. Med. Biol. 513 (2002) 397–430.
[99] M.E. Davis, The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic, Mol. Pharm. 6 (2009) 659–668.
[100] J.E. Zuckerman, I. Gritli, A. Tolcher, J.D. Heidel, D. Lim, R. Morgan, B. Chmielowski, A. Ribas, M.E. Davis, Y. Yen, Correlating animal and human phase Ia/Ib clinical data with CALAA-01, a targeted, polymer-based nanoparticle containing siRNA, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 11449–11454.
[101] T.R. Daniels, E. Bernabeu, J.A. Rodriguez, S. Patel, M. Kozman, D.A. Chiappetta, E. Holler, J.Y. Ljubimova, G. Helguera, M.L. Penichet, The transferrin receptor and the targeted delivery of therapeutic agents against cancer, Biochim. Biophys. Acta 1820 (2012) 291–317.
[102] Y. Yu, Z. Pang, W. Lu, Q. Yin, H. Gao, X. Jiang, Self-assembled polymersomes conjugated with lactoferrin as novel drug carrier for brain delivery, Pharm. Res. 29 (2012) 83–96.
[103] A. Tomas, C.E. Futter, E.R. Eden, EGF receptor trafficking: consequences for signaling and cancer, Trends Cell Biol. 24 (2014) 26–34.
[104] M.A. Sandoval, B.R. Sloat, P.D. Lansakara, A. Kumar, B.L. Rodriguez, K. Kiguchi, J. Digiovanni, Z. Cui, EGFR-targeted stearoyl gemcitabine nanoparticles show enhanced anti-tumor activity, J. Control. Release 157 (2012) 287–296.
[105] M. Marcus, H. Skaat, N. Alon, S. Margel, O. Shefi, NGF-conjugated iron oxide nanoparticles promote differentiation and outgrowth of PC12 cells, Nanoscale 7 (2014) 1058–1066.
[106] V.A. Petrenko, P.K. Jayanna, Phage protein-targeted cancer nanomedicines, FEBS Lett. 588 (2014) 341–349.
[107] E. Ruoslahti, Peptides as targeting elements and tissue penetration devices for nanoparticles, Adv. Mater. 24 (2012) 3747–3756.
[108] R. Brissette, J.K. Prendergast, N.I. Goldstein, Identification of cancer targets and therapeutics using phage display, Curr. Opin. Drug Discov. Devel. 9 (2006) 363–369.
[109] D.N. Krag, G.S. Shukla, G.P. Shen, S. Pero, T. Ashikaga, S. Fuller, D.L. Weaver, S. Burdette-Radoux, C. Thomas, Selection of tumor-binding ligands in cancer patients with phage display libraries, Cancer Res. 66 (2006) 7724–7733.
[110] J.S. Desgrosellier, D.A. Cheresh, Integrins in cancer: biological implications and therapeutic opportunities, Nat. Rev. Cancer 10 (2010) 9–22.
[111] L.W. Contois, A. Akalu, J.M. Caron, E. Tweedie, A. Cretu, T. Henderson, L. Liaw, R. Friesel, C. Vary, P.C. Brooks, Inhibition of tumor-associated alphavbeta3 integrin regulates the angiogenic switch by enhancing expression of IGFBP-4 leading to reduced melanoma growth and angiogenesis in vivo, Angiogenesis (2014).
[112] G. Mondal, S. Barui, A. Chaudhuri, The relationship between the cyclic-RGDfK ligand and alphavbeta3 integrin receptor, Biomaterials 34 (2013) 6249–6260.
[113] P.H. O'Donnell, S.D. Undevia, W.M. Stadler, T.M. Karrison, M.K. Nicholas, L. Janisch, M.J. Ratain, A phase I study of continuous infusion cilengitide in patients with solid tumors, Invest. New Drugs 30 (2012) 604–610.
[114] A. Alva, S. Slovin, S. Daignault, M. Carducci, R. Dipaola, K. Pienta, D. Agus, K. Cooney, A. Chen, D.C. Smith, M. Hussain, Phase II study of cilengitide (EMD 121974, NSC 707544) in patients with non-metastatic castration resistant prostate cancer, NCI-6735. A study by the DOD/PCF prostate cancer clinical trials consortium, Invest. New Drugs 30 (2012) 749–757.
[115] A. Carter, Integrins as target: first phase III trial launches, but questions remain, J. Natl. Cancer Inst. 102 (2010) 675–677.
[116] K. Fink, T. Mikkelsen, L.E. Nabors, P. Ravin, S.R. Plotkin, D. Schiff, C. Hicking, M. Picard, D.A. Reardon, Long-term effects of cilengitide, a novel integrin inhibitor, in recurrent glioblastoma: a randomized phase IIa study, Clin. Oncol. J 28 (2010).
[117] J. Domingo-Espin, V. Petegnief, N. de Vera, O. Conchillo-Sole, P. Saccardo, U. Unzueta, E. Vazquez, J. Cedano, L. Negro, X. Daura, H. Peluffo, A.M. Planas, A. Villaverde, N. Ferrer-Miralles, RGD-based cell ligands for cell-targeted drug delivery act as potent trophic factors, Nanomedicine 8 (2012) 1263–1266.
[118] S. Valetti, S. Mura, M. Noiray, S. Arpicco, F. Dosio, J. Vergnaud, D. Desmaele, B. Stella, P. Couvreur, Peptide conjugation: before or after nanoparticle formation? Bioconjug. Chem. 25 (2014) 1971–1983.
[119] A. Ray, N. Larson, D.B. Pike, M. Gruner, S. Naik, H. Bauer, A. Malugin, K. Greish, H. Ghandehari, Comparison of active and passive targeting of docetaxel for prostate cancer therapy by HPMA copolymer-RGDfK conjugates, Mol. Pharm. 8 (2011) 1090–1099.
[120] A. Eldar-Boock, K. Miller, J. Sanchis, R. Lupu, M.J. Vicent, R. Satchi-Fainaro, Integrin-assisted drug delivery of nano-scaled polymer therapeutics bearing paclitaxel, Biomaterials 32 (2011) 3862–3874.
[121] D. Zhou, G. Zhang, Z. Gan, c(RGDfK) decorated micellar drug delivery system for intravesical instilled chemotherapy of superficial bladder cancer, J. Control. Release 169 (2013) 204–210.
[122] F. Wang, L. Chen, R. Zhang, Z. Chen, L. Zhu, RGD peptide conjugated liposomal drug delivery system for enhance therapeutic efficacy in treating bone metastasis from prostate cancer, J. Control. Release 196 (2014) 222–233.
[123] C. Puig-Saus, L.A. Rojas, E. Laborda, A. Figueras, R. Alba, C. Fillat, R. Alemany, iRGD tumor-penetrating peptide-modified oncolytic adenovirus shows enhanced tumor transduction, intratumoral dissemination and antitumor efficacy, Gene Ther. 21 (2014) 767–774.
[124] K.N. Sugahara, G.B. Braun, T.H. de Mendoza, V.R. Kotamraju, R.P. French, A.M. Lowy, T. Teesalu, E. Ruoslahti, Tumor-penetrating iRGD peptide inhibits metastasis, Mol. Cancer Ther. (2014).
[125] E. Ruoslahti, Specialization of tumour vasculature, Nat. Rev. Cancer 2 (2002) 83–90.
[126] T. Teesalu, K.N. Sugahara, V.R. Kotamraju, E. Ruoslahti, C-end rule peptides mediate neuropilin-1-dependent cell, vascular, and tissue penetration, Proc, Natl. Acad. Sci. U. S. A. 106 (2009) 16157–16162.
[127] Z. Zhu, C. Xie, Q. Liu, X. Zhen, X. Zheng, W. Wu, R. Li, Y. Ding, X. Jiang, B. Liu, The effect of hydrophilic chain length and iRGD on drug delivery from poly(epsilon-caprolactone)-poly(N-vinylpyrrolidone) nanoparticles, Biomaterials 32 (2011) 9525–9535.
[128] K. Wang, X. Zhang, Y. Liu, C. Liu, B. Jiang, Y. Jiang, Tumor penetrability and anti-angiogenesis using iRGD-mediated delivery of doxorubicin-polymer conjugates, Biomaterials 35 (2014) 8735–8747.
[129] P. Laakkonen, K. Porkka, J.A. Hoffman, E. Ruoslahti, A tumor-homing peptide with a targeting specificity related to lymphatic vessels, Nat. Med. 8 (2002) 751–755.
[130] V. Fogal, L. Zhang, S. Krajewski, E. Ruoslahti, Mitochondrial/cell-surface protein p32/gC1qR as a molecular target in tumor cells and tumor stroma, Cancer Res. 68 (2008) 7210–7218.
[131] P.P. Karmali, V.R. Kotamraju, M. Kastantin, M. Black, D. Missirlis, M. Tirrell, E. Ruoslahti, Targeting of albumin-embedded paclitaxel nanoparticles to tumors, Nanomedicine 5 (2009) 73–82.
[132] P. Laakkonen, M.E. Akerman, H. Biliran, M. Yang, F. Ferrer, T. Karpanen, R.M. Hoffman, E. Ruoslahti, Antitumor activity of a homing peptide that targets tumor lymphatics and tumor cells, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 9381–9386.
[133] Z. Wang, Y. Yu, J. Ma, H. Zhang, H. Zhang, X. Wang, J. Wang, X. Zhang, Q. Zhang, LyP-1 modification to enhance delivery of artemisinin or fluorescent probe loaded polymeric micelles to highly metastatic tumor and its lymphatics, Mol. Pharm. 9 (2012) 2646–2657.
[134] K. Porkka, P. Laakkonen, J.A. Hoffman, M. Bernasconi, E. Ruoslahti, A fragment of the HMGN2 protein homes to the nuclei of tumor cells and tumor endothelial cells in vivo, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 7444–7449.
[135] G.R. Reddy, M.S. Bhojani, P. McConville, J. Moody, B.A. Moffat, D.E. Hall, G. Kim, Y.E. Koo, M.J. Woolliscroft, J.V. Sugai, T.D. Johnson, M.A. Philbert, R. Kopelman, A. Rehemtulla, B.D. Ross, Vascular targeted nanoparticles for imaging and treatment of brain tumors, Clin. Cancer Res. 12 (2006) 6677–6686.
[136] I. Winer, S. Wang, Y.E. Lee, W. Fan, Y. Gong, D. Burgos-Ojeda, G. Spahlinger, R. Kopelman, R.J. Buckanovich, F3-targeted cisplatin-hydrogel nanoparticles as an effective therapeutic that targets both murine and human ovarian tumor endothelial cells in vivo, Cancer Res. 70 (2010) 8674–8683.
[137] T.R. Pearce, K. Shroff, E. Kokkoli, Peptide targeted lipid nanoparticles for anticancer drug delivery, Adv. Mater. 24 (3803-3822) (2012) 3710.
[138] Y.G. Assaraf, C.P. Leamon, J.A. Reddy, The folate receptor as a rational therapeutic target for personalized cancer treatment, Drug Resist. Updat. 17 (2014) 89–95.

[139] W.T. Chen, S.T. Kang, J.L. Lin, C.H. Wang, R.C. Chen, C.K. Yeh, Targeted tumor theranostics using folate-conjugated and camptothecin-loaded acoustic nanodroplets in a mouse xenograft model, Biomaterials 53 (2015) 699–708.
[140] I.R. Vlahov, C.P. Leamon, Engineering folate-drug conjugates to target cancer: from chemistry to clinic, Bioconjug. Chem. 23 (2012) 1357–1369.
[141] R.W. Naumann, R.L. Coleman, R.A. Burger, E.A. Sausville, E. Kutarska, S.A. Ghamande, N.Y. Gabrail, S.E. Depasquale, E. Nowara, L. Gilbert, R.H. Gersh, M.G. Teneriello, W.A. Harb, P.A. Konstantinopoulos, R.T. Penson, J.T. Symanowski, C.D. Lovejoy, C.P. Leamon, D.E. Morgenstern, R.A. Messmann, PRECEDENT: a randomized phase II trial comparing vintafolide (EC145) and pegylated liposomal doxorubicin (PLD) in combination versus PLD alone in patients with platinum-resistant ovarian cancer, J. Clin. Oncol. 31 (2013) 4400–4406.
[142] P.M. Lorusso, M.J. Edelman, S.L. Bever, K.M. Forman, M. Pilat, M.F. Quinn, J. Li, E.I. Heath, L.M. Malburg, P.J. Klein, C.P. Leamon, R.A. Messmann, E.A. Sausville, Phase I study of folate conjugate EC145 (Vintafolide) in patients with refractory solid tumors, J. Clin. Oncol. 30 (2012) 4011–4016.
[143] A.O. Saeed, J.P. Magnusson, E. Moradi, M. Soliman, W. Wang, S. Stolnik, K.J. Thurecht, S.M. Howdle, C. Alexander, Modular construction of multifunctional bioresponsive cell-targeted nanoparticles for gene delivery, Bioconjug. Chem. 22 (2011) 156–168.
[144] A.T. Wang, D.S. Liang, Y.J. Liu, X.R. Qi, Roles of ligand and TPGS of micelles in regulating internalization, penetration and accumulation against sensitive or resistant tumor and therapy for multidrug resistant tumors, Biomaterials 53 (2015) 160–172.
[145] Y. Zhou, H. Wang, C. Wang, Y. Li, W. Lu, S. Chen, J. Luo, Y. Jiang, J. Chen, Receptor-mediated, tumor-targeted gene delivery using folate-terminated polyrotaxanes, Mol. Pharm. 9 (2012) 1067–1076.
[146] T. Yang, B. Li, S. Qi, Y. Liu, Y. Gai, P. Ye, G. Yang, W. Zhang, P. Zhang, X. He, W. Li, Z. Zhang, G. Xiang, C. Xu, Co-delivery of doxorubicin and Bmi1 siRNA by folate receptor targeted liposomes exhibits enhanced anti-tumor effects in vitro and in vivo, Theranostics 4 (2014) 1096–1111.
[147] Y. Shi, C. Su, W. Cui, H. Li, L. Liu, B. Feng, M. Liu, R. Su, L. Zhao, Gefitinib loaded folate decorated bovine serum albumin conjugated carboxymethyl-beta-cyclodextrin nanoparticles enhance drug delivery and attenuate autophagy in folate receptor-positive cancer cells, J. Nanobiotechnol. 12 (2014) 43.
[148] H. Li, Y. Lu, L. Piao, J. Wu, S. Liu, G. Marcucci, M. Ratnam, R.J. Lee, Targeting human clonogenic acute myelogenous leukemia cells via folate conjugated liposomes combined with receptor modulation by all-trans retinoic acid, Int. J. Pharm. 402 (2010) 57–63.
[149] P.M. Valencia, M.H. Hanewich-Hollatz, W. Gao, F. Karim, R. Langer, R. Karnik, O.C. Farokhzad, Effects of ligands with different water solubilities on self-assembly and properties of targeted nanoparticles, Biomaterials 32 (2011) 6226–6233.
[150] Z. Tang, D. Li, H. Sun, X. Guo, Y. Chen, S. Zhou, Quantitative control of active targeting of nanocarriers to tumor cells through optimization of folate ligand density, Biomaterials 35 (2014) 8015–8027.
[151] W. Zhao, M. Cai, H. Xu, J. Jiang, H. Wang, A single-molecule force spectroscopy study of the interactions between lectins and carbohydrates on cancer and normal cells, Nanoscale 5 (2013) 3226–3229.
[152] J. Liu, W.D. Gray, M.E. Davis, Y. Luo, Peptide- and saccharide-conjugated dendrimers for targeted drug delivery: a concise review, Interface Focus 2 (2012) 307–324.
[153] R. Yang, F. Meng, S. Ma, F. Huang, H. Liu, Z. Zhong, Galactose-decorated cross-linked biodegradable poly(ethylene glycol)-b-poly(epsilon-caprolactone) block copolymer micelles for enhanced hepatoma-targeting delivery of paclitaxel, Biomacromolecules 12 (2011) 3047–3055.
[154] Y. Zhong, W. Yang, H. Sun, R. Cheng, F. Meng, C. Deng, Z. Zhong, Ligand-directed reduction-sensitive shell-sheddable biodegradable micelles actively deliver doxorubicin into the nuclei of target cancer cells, Biomacromolecules 14 (2013) 3723–3730.
[155] L.Z. He, J. Weidlick, C. Sisson, H.C. Marsh, T. Keler, Toll-like receptor agonists shape the immune responses to a mannose receptor-targeted cancer vaccine, Cell. Mol. Immunol. (2014).
[156] J.M. Silva, E. Zupancic, G. Vandermeulen, V.G. Oliveira, A. Salgado, M. Videira, M. Gaspar, L. Graca, V. Preat, H.F. Florindo, In vivo delivery of peptides and Toll-like receptor ligands by mannose-functionalized polymeric nanoparticles induces prophylactic and therapeutic anti-tumor immune responses in a melanoma model, J. Control. Release 198C (2014) 91–103.
[157] S. Mizrahy, D. Peer, Polysaccharides as building blocks for nanotherapeutics, Chem. Soc. Rev. 41 (2012) 2623–2640.
[158] S. Seton-Rogers, Metastasis: multitasking hyaluronic acid, Nat. Rev. Cancer 12 (2012) 228.
[159] G. Saravanakumar, D.G. Jo, J.H. Park, Polysaccharide-based nanoparticles: a versatile platform for drug delivery and biomedical imaging, Curr. Med. Chem. 19 (2012) 3212–3229.
[160] H.Y. Yoon, H. Koo, K.Y. Choi, S.J. Lee, K. Kim, I.C. Kwon, J.F. Leary, K. Park, S.H. Yuk, J.H. Park, K. Choi, Tumor-targeting hyaluronic acid nanoparticles for photodynamic imaging and therapy, Biomaterials 33 (2012) 3980–3989.
[161] H.S. Han, J. Lee, H.R. Kim, S.Y. Chae, M. Kim, G. Saravanakumar, H.Y. Yoon, D.G. You, H. Ko, K. Kim, I.C. Kwon, J.C. Park, J.H. Park, Robust PEGylated hyaluronic acid nanoparticles as the carrier of doxorubicin: mineralization and its effect on tumor targetability in vivo, J. Control. Release 168 (2013) 105–114.
[162] J. Huang, H. Zhang, Y. Yu, Y. Chen, D. Wang, G. Zhang, G. Zhou, J. Liu, Z. Sun, D. Sun, Y. Lu, Y. Zhong, Biodegradable self-assembled nanoparticles of poly (D,L-lactide-co-glycolide)/hyaluronic acid block copolymers for target delivery of docetaxel to breast cancer, Biomaterials 35 (2014) 550–566.
[163] Y. Zhong, F. Meng, C. Deng, Z. Zhong, Ligand-directed active tumor-targeting polymeric nanoparticles for cancer chemotherapy, Biomacromolecules 15 (2014) 1955–1969.
[164] Q. Tian, X.H. Wang, W. Wang, C.N. Zhang, P. Wang, Z. Yuan, Self-assembly and liver targeting of sulfated chitosan nanoparticles functionalized with glycyrrhetinic acid, Nanomedicine 8 (2012) 870–879.
[165] L. Shi, C. Tang, C. Yin, Glycyrrhizin-modified O-carboxymethyl chitosan nanoparticles as drug vehicles targeting hepatocellular carcinoma, Biomaterials 33 (2012) 7594–7604.
[166] E. Sarafraz-Yazdi, M.R. Pincus, J. Michl, Tumor-targeting peptides and small molecules as anti-cancer agents to overcome drug resistance, Curr. Med. Chem. 21 (2014) 1618–1630.
[167] K.P. Maresca, S.M. Hillier, F.J. Femia, D. Keith, C. Barone, J.L. Joyal, C.N. Zimmerman, A.P. Kozikowski, J.A. Barrett, W.C. Eckelman, J.W. Babich, A series of halogenated heterodimeric inhibitors of prostate specific membrane antigen (PSMA) as radiolabeled probes for targeting prostate cancer, J. Med. Chem. 52 (2009) 347–357.
[168] Y. Chen, S. Dhara, S.R. Banerjee, Y. Byun, M. Pullambhatla, R.C. Mease, M.G. Pomper, A low molecular weight PSMA-based fluorescent imaging agent for cancer, Biochem. Biophys. Res. Commun. 390 (2009) 624–629.
[169] V. Sanna, G. Pintus, A.M. Roggio, S. Punzoni, A.M. Posadino, A. Arca, S. Marceddu, P. Bandiera, S. Uzzau, M. Sechi, Targeted biocompatible nanoparticles for the delivery of (−)-epigallocatechin 3-gallate to prostate cancer cells, J. Med. Chem. 54 (2011) 1321–1332.
[170] S.S. Chandran, S.R. Banerjee, R.C. Mease, M.G. Pomper, S.R. Denmeade, Characterization of a targeted nanoparticle functionalized with a urea-based inhibitor of prostate-specific membrane antigen (PSMA), Cancer Biol. Ther. 7 (2008) 974–982.
[171] V. Sanna, M. Sechi, Nanoparticle therapeutics for prostate cancer treatment, Nanomedicine 8 (Suppl. 1) (2012) S31–S36.
[172] V. Sanna, N. Pala, M. Sechi, Targeted therapy using nanotechnology: focus on cancer, Int. J. Nanomed. 9 (2014) 467–483.
[173] W. Huang, K. Dolmer, P.G. Gettins, NMR solution structure of complement-like repeat CR8 from the low density lipoprotein receptor-related protein, J. Biol. Chem. 274 (1999) 14130–14136.
[174] J. Gliemann, Receptors of the low density lipoprotein (LDL) receptor family in man. Multiple functions of the large family members via interaction with complex ligands, Biol. Chem. 379 (1998) 951–964.
[175] J. Tschopp, T.E. Mollnes, Antigenic crossreactivity of the alpha subunit of complement component C8 with the cysteine-rich domain shared by complement component C9 and low density lipoprotein receptor, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 4223–4227.
[176] J. Silverman, Q. Liu, A. Bakker, W. To, A. Duguay, B.M. Alba, R. Smith, A. Rivas, P. Li, H. Le, E. Whitehorn, K.W. Moore, C. Swimmer, V. Perlroth, M. Vogt, J. Kolkman, W.P. Stemmer, Multivalent avimer proteins evolved by exon shuffling of a family of human receptor domains, Nat. Biotechnol. 23 (2005) 1556–1561.
[177] R. Smith, A. Duguay, A. Bakker, P. Li, J. Weiszmann, M.R. Thomas, B.M. Alba, X. Wu, J. Gupte, L. Yang, J. Stevens, A. Hamburger, S. Smith, J. Chen, R. Komorowski, K.W. Moore, M.M. Veniant, Y. Li, FGF21 can be mimicked in vitro and in vivo by a novel anti-FGFR1c/beta-Klotho bispecific protein, PLoS ONE 8 (2013) e61432.
[178] L. Xu, P. Aha, K. Gu, R.G. Kuimelis, M. Kurz, T. Lam, A.C. Lim, H. Liu, P.A. Lohse, L. Sun, S. Weng, R.W. Wagner, D. Lipovsek, Directed evolution of high-affinity antibody mimics using mRNA display, Chem. Biol. 9 (2002) 933–942.
[179] V. Ramamurthy, S.R. Krystek Jr., A. Bush, A. Wei, S.L. Emanuel, R. Das Gupta, A. Janjua, L. Cheng, M. Murdock, B. Abramczyk, D. Cohen, Z. Lin, P. Morin, J.H. Davis, M. Dabritz, D.C. McLaughlin, K.A. Russo, G. Chao, M.C. Wright, V.A. Jenny, L.J. Engle, E. Furfine, S. Sheriff, Structures of adnectin/protein complexes reveal an expanded binding footprint, Structure 20 (2012) 259–269.
[180] S.P. Dineen, L.A. Sullivan, A.W. Beck, A.F. Miller, J.G. Carbon, R. Mamluk, H. Wong, R.A. Brekken, The Adnectin CT-322 is a novel VEGF receptor 2 inhibitor that decreases tumor burden in an orthotopic mouse model of pancreatic cancer, BMC Cancer 8 (2008) 352.
[181] R.E. Iacob, G. Chen, J. Ahn, S. Houel, H. Wei, J. Mo, L. Tao, D. Cohen, D. Xie, Z. Lin, P.E. Morin, M.L. Doyle, A.A. Tymiak, J.R. Engen, The influence of adnectin binding on the extracellular domain of epidermal growth factor receptor, J. Am. Soc. Mass. Spectrom. 25 (2014) 2093–2102.
[182] A.W. Tolcher, C.J. Sweeney, K. Papadopoulos, A. Patnaik, E.G. Chiorean, A.C. Mita, K. Sankhala, E. Furfine, J. Gokemeijer, L. Iacono, C. Eaton, B.A. Silver, M. Mita, Phase I and pharmacokinetic study of CT-322 (BMS-844203), a targeted Adnectin inhibitor of VEGFR-2 based on a domain of human fibronectin, Clin. Cancer Res. 17 (2011) 363–371.
[183] Z. Qian, P.G. Dougherty, D. Pei, Monitoring the cytosolic entry of cell-penetrating peptides using a pH-sensitive fluorophore, Chem. Commun. (Camb.) (2015).
[184] M. Rapoport, H. Lorberboum-Galski, TAT-based drug delivery system – new directions in protein delivery for new hopes? Expert Opin. Drug Deliv. 6 (2009) 453–463.

[185] A. Apte, E. Koren, A. Koshkaryev, V.P. Torchilin, Doxorubicin in TAT peptide-modified multifunctional immunoliposomes demonstrates increased activity against both drug-sensitive and drug-resistant ovarian cancer models, Cancer Biol. Ther. 15 (2014) 69–80.
[186] S. Yamano, J. Dai, S. Hanatani, K. Haku, T. Yamanaka, M. Ishioka, T. Takayama, C. Yuvienco, S. Khapli, A.M. Moursi, J.K. Montclare, Long-term efficient gene delivery using polyethylenimine with modified Tat peptide, Biomaterials 35 (2014) 1705–1715.
[187] Y. Lei, H. Tang, L. Yao, R. Yu, M. Feng, B. Zou, Applications of mesenchymal stem cells labeled with Tat peptide conjugated quantum dots to cell tracking in mouse body, Bioconjug. Chem. 19 (2008) 421–427.
[188] V.A. Sethuraman, Y.H. Bae, TAT peptide-based micelle system for potential active targeting of anti-cancer agents to acidic solid tumors, J. Control. Release 118 (2007) 216–224.
[189] E.S. Lee, Z. Gao, D. Kim, K. Park, I.C. Kwon, Y.H. Bae, Super pH-sensitive multifunctional polymeric micelle for tumor pH(e) specific TAT exposure and multidrug resistance, J. Control. Release 129 (2008) 228–236.
[190] S. Muro, Challenges in design and characterization of ligand-targeted drug delivery systems, J. Control. Release 164 (2012) 125–137.
[191] G.T. Noble, J.F. Stefanick, J.D. Ashley, T. Kiziltepe, B. Bilgicer, Ligand-targeted liposome design: challenges and fundamental considerations, Trends Biotechnol. 32 (2014) 32–45.
[192] B. Wang, C.V. Galliford, P.S. Low, Guiding principles in the design of ligand-targeted nanomedicines, Nanomedicine (Lond.) 9 (2014) 313–330.
[193] D. Ling, H. Xia, W. Park, M.J. Hackett, C. Song, K. Na, K.M. Hui, T. Hyeon, pH-sensitive nanoformulated triptolide as a targeted therapeutic strategy for hepatocellular carcinoma, ACS Nano 8 (2014) 8027–8039.
[194] E.S. Lee, K. Na, Y.H. Bae, Super pH-sensitive multifunctional polymeric micelle, Nano Lett. 5 (2005) 325–329.
[195] L. Han, Y. Guo, H. Ma, X. He, Y. Kuang, N. Zhang, E. Lim, W. Zhou, C. Jiang, Acid active receptor-specific peptide ligand for in vivo tumor-targeted delivery, Small 9 (2013) 3647–3658.
[196] L. Han, R. Huang, J. Li, S. Liu, S. Huang, C. Jiang, Plasmid pORF-hTRAIL and doxorubicin co-delivery targeting to tumor using peptide-conjugated polyamidoamine dendrimer, Biomaterials 32 (2011) 1242–1252.
[197] P.R. Leroueil, S. Hong, A. Mecke, J.R. Baker Jr., B.G. Orr, M.M. Banaszak Holl, Nanoparticle interaction with biological membranes: does nanotechnology present a Janus face? Acc. Chem. Res. 40 (2007) 335–342.
[198] O. Harush-Frenkel, N. Debotton, S. Benita, Y. Altschuler, Targeting of nanoparticles to the clathrin-mediated endocytic pathway, Biochem. Biophys. Res. Commun. 353 (2007) 26–32.
[199] R. Bausinger, K. von Gersdorff, K. Braeckmans, M. Ogris, E. Wagner, C. Brauchle, A. Zumbusch, The transport of nanosized gene carriers unraveled by live-cell imaging, Angew. Chem. Int. Ed. Engl. 45 (2006) 1568–1572.
[200] Y. Shen, Z. Zhou, M. Sui, J. Tang, P. Xu, E.A. Van Kirk, W.J. Murdoch, M. Fan, M. Radosz, Charge-reversal polyamidoamine dendrimer for cascade nuclear drug delivery, Nanomedicine (Lond.) 5 (2010) 1205–1217.
[201] W. Wu, J. Wang, Z. Lin, X. Li, J. Li, Tumor-acidity activated surface charge-conversion of polymeric nanocarriers for enhanced cell adhesion and targeted drug release, Macromol. Rapid Commun. 35 (2014) 1679–1684.
[202] H. Huang, Y. Li, Z. Sa, Y. Sun, Y. Wang, J. Wang, A smart drug delivery system from charge-conversion polymer-drug conjugate for enhancing tumor therapy and tunable drug release, Macromol. Biosci. 14 (2014) 485–490.
[203] Y.Y. Yuan, C.Q. Mao, X.J. Du, J.Z. Du, F. Wang, J. Wang, Surface charge switchable nanoparticles based on zwitterionic polymer for enhanced drug delivery to tumor, Adv. Mater. 24 (2012) 5476–5480.
[204] P. Xu, E.A. Van Kirk, Y. Zhan, W.J. Murdoch, M. Radosz, Y. Shen, Targeted charge-reversal nanoparticles for nuclear drug delivery, Angew. Chem. Int. Ed. Engl. 46 (2007) 4999–5002.
[205] R. Savic, L. Luo, A. Eisenberg, D. Maysinger, Micellar nanocontainers distribute to defined cytoplasmic organelles, Science 300 (2003) 615–618.
[206] D.J. Reijngoud, J.M. Tager, The permeability properties of the lysosomal membrane, Biochim. Biophys. Acta 472 (1977) 419–449.
[207] J.Z. Du, X.J. Du, C.Q. Mao, J. Wang, Tailor-made dual pH-sensitive polymer-doxorubicin nanoparticles for efficient anticancer drug delivery, J. Am. Chem. Soc. 133 (2011) 17560–17563.
[208] C. Ding, J. Gu, X. Qu, Z. Yang, Preparation of multifunctional drug carrier for tumor-specific uptake and enhanced intracellular delivery through the conjugation of weak acid labile linker, Bioconjug. Chem. 20 (2009) 1163–1170.
[209] J. Gu, W.P. Cheng, J. Liu, S.Y. Lo, D. Smith, X. Qu, Z. Yang, pH-triggered reversible “stealth” polycationic micelles, Biomacromolecules 9 (2008) 255–262.
[210] Y. Huang, Y. Li, Drug delivery and reversal of MDR, Mol. Pharm. 11 (2014) 2493–2494.
[211] A. Shapira, Y.D. Livney, H.J. Broxterman, Y.G. Assaraf, Nanomedicine for targeted cancer therapy: towards the overcoming of drug resistance, Drug Resist. Updat. 14 (2011) 150–163.
[212] H. Park, W. Park, K. Na, Doxorubicin loaded singlet-oxygen producible polymeric micelle based on chlorine e6 conjugated pluronic F127 for overcoming drug resistance in cancer, Biomaterials 35 (2014) 7963–7969.
[213] E.V. Batrakova, A.V. Kabanov, Pluronic block copolymers: evolution of drug delivery concept from inert nanocarriers to biological response modifiers, J. Control. Release 130 (2008) 98–106.
[214] A.V. Kabanov, E.V. Batrakova, V.Y. Alakhov, Pluronic block copolymers for overcoming drug resistance in cancer, Adv. Drug Deliv. Rev. 54 (2002) 759–779.
[215] X. Dong, R.J. Mumper, Nanomedicinal strategies to treat multidrug-resistant tumors: current progress, Nanomedicine (Lond.) 5 (2010) 597–615.
[216] K. Cohen, R. Emmanuel, E. Kisin-Finfer, D. Shabat, D. Peer, Modulation of drug resistance in ovarian adenocarcinoma using chemotherapy entrapped in hyaluronan-grafted nanoparticle clusters, ACS Nano 8 (2014) 2183–2195.
[217] E.S. Lee, K. Na, Y.H. Bae, Doxorubicin loaded pH-sensitive polymeric micelles for reversal of resistant MCF-7 tumor, J. Control. Release 103 (2005) 405–418.
[218] S.K. Sahoo, W. Ma, V. Labhasetwar, Efficacy of transferrin-conjugated paclitaxel-loaded nanoparticles in a murine model of prostate cancer, Int. J. Cancer 112 (2004) 335–340.
[219] R. Suzuki, T. Takizawa, Y. Kuwata, M. Mutoh, N. Ishiguro, N. Utoguchi, A. Shinohara, M. Eriguchi, H. Yanagie, K. Maruyama, Effective anti-tumor activity of oxaliplatin encapsulated in transferrin-PEG-liposome, Int. J. Pharm. 346 (2008) 143–150.
[220] H. Matsuo, M. Wakasugi, H. Takanaga, H. Ohtani, M. Naito, T. Tsuruo, Y. Sawada, Possibility of the reversal of multidrug resistance and the avoidance of side effects by liposomes modified with MRK-16, a monoclonal antibody to P-glycoprotein, J. Control. Release 77 (2001) 77–86.
[221] F. Danhier, A. Le Breton, V. Preat, RGD-based strategies to target alpha(v) beta(3) integrin in cancer therapy and diagnosis, Mol. Pharm. 9 (2012) 2961–2973.