Journal of Controlled Release 148 (2010) 135–146

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review

To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery

Fabienne Danhier [a], Olivier Feron [b], Véronique Préat [a,*]

[a] Université Catholique de Louvain, Louvain Drug Research Institute, Unit of Pharmaceutics, UCL-FARG 7320, Avenue E. Mounier, B-1200, Brussels, Belgium
[b] Université Catholique de Louvain, Pole of Pharmacology and Therapeutics, Institute of Experimental and Clinical Research, UCL-FATH 5349, Avenue E. Mounier, B-1200, Brussels, Belgium

ARTICLE INFO

Article history:
Received 31 May 2010
Accepted 10 August 2010
Available online 24 August 2010

Keywords:
Active targeting
Passive targeting
Enhanced Permeability and Retention effect
Nanocarriers

ABSTRACT

Because of the particular characteristics of the tumor microenvironment and tumor angiogenesis, it is possible to design drug delivery systems that specifically target anti-cancer drugs to tumors. Most of the conventional chemotherapeutic agents have poor pharmacokinetics profiles and are distributed non-specifically in the body leading to systemic toxicity associated with serious side effects. Therefore, the development of drug delivery systems able to target the tumor site is becoming a real challenge that is currently addressed. Nanomedicine can reach tumor passively through the leaky vasculature surrounding the tumors by the Enhanced Permeability and Retention effect whereas ligands grafted at the surface of nanocarriers allow active targeting by binding to the receptors overexpressed by cancer cells or angiogenic endothelial cells.

This review is divided into two parts: the first one describes the tumor microenvironment and the second one focuses on the exploitation and the understanding of these characteristics to design new drug delivery systems targeting the tumor. Delivery of conventional chemotherapeutic anti-cancer drugs is mainly discussed.

Contents

1. Introduction

Cancer is a leading cause of death around the world. The World Health Organization estimates that 84 million people will die of cancer between 2005 and 2015. For effective cancer therapy, it is necessary to improve our knowledge of cancer physiopathology, discover new anti-cancer drugs and develop novel biomedical

* Corresponding author. Université Catholique de Louvain, Louvain Drug Research Institute, Unité de pharmacie galénique, Avenue Mounier UCL 7320, B-1200 Brussels, Belgium. Tel.: +32 2 7647320; fax: +32 2 7647398.
E-mail address: veronique.preat@uclouvain.be (V. Préat).

0168-3659/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2010.08.027

technologies. Currently, the cancer therapy has become a multidisciplinary challenge requiring close collaboration among clinicians, biological and materials scientists, and biomedical engineers. Conventional chemotherapeutic agents are distributed non-specifically in the body affecting both normal and tumoral cells. Given the potency of modern pharmacological agents, tissue selectivity is a major issue. Hence, the dose achievable within the solid tumor is limited resulting in suboptimal treatment due to excessive toxicities. The ultimate goal of cancer therapeutics is to increase the survival time and the quality of life of the patient by reducing the systemic toxicity of chemotherapy [1].The idea of exploiting vascular abnormalities of tumors, avoiding penetration into normal tissue interstitium while allowing access to tumors, becomes particularly attractive. In this context, the tumor targeting of nanomedicine-based therapeutics has emerged as one approach to overcome the lack of specificity of conventional chemotherapeutic agents [2,3].

This concept dates back to 1906 when Ehrlich first imagined the "magic bullet" [4]. The challenge of the targeting is triple: (i) to find the proper target for a particular disease; (ii) to find the drug that effectively treats this disease and (iii) to find how to carry the drug. The specific tumor targeting of nanocarriers leads to better profiles of pharmacokinetics and pharmacodynamics, controlled and sustained release of drugs, an improved specificity, an increased internalization and intracellular delivery and, more importantly, a lower systemic toxicity. The tumor targeting consists in "passive targeting" and "active targeting"; however, the active targeting process cannot be separated from the passive because it occurs only after passive accumulation in tumors [5].

New moleculary targeted anti-cancer agents currently used in clinical trials illustrate the success of the targeting concept (imatinib mesylate (Gleevec®), gefitinib (Iressa®), trastuzumab (Herceptin®), and cetuximab (C225, Erbitux®). Alternatively, existing anti-cancer agents can be more effective by using nanomedicines (the medical application of nanotechnology). The European Science Foundation's Forward Look on Nanomedicine defined nanomedicines as «nanometer size scale complex systems, consisting of at least two components, one of which being the active ingredient». Protecting drug from the degradation, nanocarriers have to be able to target a drug to the tumor site, reducing damage to normal tissue (Table 1). The development of nanocarriers for poorly soluble drugs is very interesting because a large proportion of new drug candidate emerging from high throughput screening are poorly-water soluble drugs which are also poorly absorbed and which present a low bioavailability. The representations of the most currently used in preclinical and clinical tumor-targeted nanomedicines are illustrated in Fig. 1.

Nanoparticles (Fig. 1A) are solid and spherical structures, ranging around 100 nm in size, in which drugs are encapsulated within the polymeric matrix. We distinguish "nanospheres" in which the drug is dispersed throughout the particles and "nanocapsules" in which the drug is entrapped in a cavity surrounded by a polymer membrane [6]. They can be PEGylated and grafted with targeting ligands (Fig. 1B). Polymeric micelles (Fig. 1A) are arranged in a spheroidal structure

Table 1
Goals and specifications of targeted nanoscale drug delivery system.

1. Increase drug concentration in the tumor through:
(a) passive targeting
(b) active targeting
2. Decrease drug concentration in normal tissue
3. Improve phamacokinetics and pharmacodynamics profiles
4. Improve the solubility of drug to allow intravenous administration
5. Release a minimum of drug during transit
6. Release a maximum of drug at the targeted site
7. Increase drug stability to reduce drug degradation
8. Improve internalization and intracellular delivery
9. Biocompatible and biodegradable

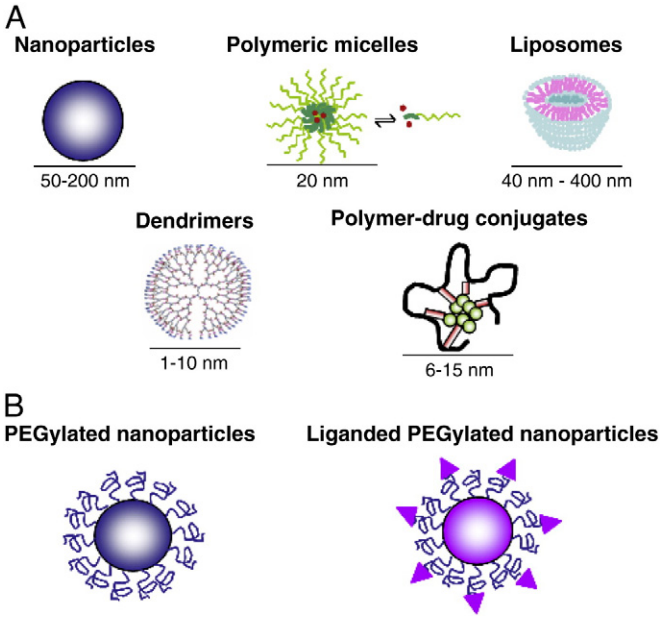

Fig. 1. Nanomedicine in drug delivery. A. Types of nanocarriers currently described in preclinical and clinical studies. B. Schematic representation of PEGylation and ligand grafting.

with hydrophobic core which increases the solubility of poorly-water soluble drugs, and the hydrophilic corona which allows a long circulation time of the drug by preventing the interactions between the core and the blood components. These systems are dynamic and have a size usually below 50 nm [2]. Liposomes (Fig. 1A) are closed spherical vesicles formed by one or several phospholipid bilayers surrounding an aqueous core in which drugs can be entrapped. They can be also PEGylated and grafted with targeting ligands [7]. Dendrimers (Fig. 1A) are highly branched macromolecules with controlled three-dimensional architecture. Polymers grow from a central core by a series of polymerisation reactions. Drugs are attached to surface groups by chemical modifications [8]. Polymer–drug conjugates (Fig. 1A) are polymeric macromolecules constituted by a polymer backbone on which drugs are conjugated via linker regions. They can be grafted with targeting ligands [9].

The common method to protect nanocarriers from the reticulo–endothelial system consists of coating the surface of the particles with polyethylene glycol (PEG), a procedure called PEGylation (Fig. 1B). To contribute to the "stealth" characteristics of PEGylated nanoparticles, there are three important factors, (i) the molecular weight of the PEG chain, (ii) the surface chain density and (iii) the conformation. The coating of PEG chains to the surface of nanoparticles results in an increase in the blood circulation half-life by several orders of magnitude. By creating a hydrophilic protective layer around the nanoparticles, steric repulsion forces repel the absorption of opsonin proteins, thereby blocking and delaying the opsonization process [10].

2. Tumor microenvironment

In cancer therapy, the tumor microenvironment is one of many areas which are studied to design new therapies. More precisely, the knowledge and the understanding of the tumor microenvironment allow researchers to elaborate different therapeutic strategies, based on numerous differences compared with normal tissue including vascular abnormalities, oxygenation, perfusion, pH and metabolic states. Here, the differences in terms of morphology of tumor vasculature and the pH will be particularly described as they are the more relevant characteristics for the design of nanocarriers as tumor-targeted drug delivery systems.

2.1. Angiogenesis in cancer

Angiogenesis is defined as the formation of new blood vessels from existing ones. For solid tumors (1–2 mm^3), oxygen and nutrients can reach the center of the tumor by simple diffusion. Because of their non-functional or non-existent vasculature, non-angiogenic tumors are highly dependent on their microenvironment for oxygen and the supply of nutrients. When tumors reach 2 mm^3, a state of cellular hypoxia begins, initiating angiogenesis. Angiogenesis is regulated by a fine balance of activators and inhibitors [11]. In the angiogenesis process, five phases can be distinguished: 1. endothelial cell activation, 2. basement membrane degradation, 3. endothelial cell migration, 4. vessel formation, and 5. angiogenic remodeling. Hypoxia increases cellular hypoxia inducible factor (HIF) transcription, leading to upregulation of pro-angiogenic proteins such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) or tumor necrosis factor-α (TNF-α) [12]. Activated endothelial cells express the dimeric transmembrane integrin $\alpha_v\beta_3$, which interacts with extracellular matrix proteins (vibronectin, fibronectin, a.o.) and regulates the migration of the endothelial cell through the extracellular matrix during vessel formation [13]. The activated endothelial cells synthesize proteolytic enzymes, such as matrix metalloproteinases, used to degrade the basement membrane and the extracellular matrix. The inner layer of endothelial cells undergoes apoptosis leading to formation of the vessel lumen. Immature vasculature undergoes extensive remodeling during which the vessels are stabilized by pericytes and smooth-muscle cells. This step is often incomplete resulting in irregular shaped, dilated and tortuous tumor blood vessels [14]. This ability of tumors to progress from a non-angiogenic to angiogenic phenotype (called the "angiogenic switch") is central to progression of cancer and allows the dissemination of cancer cells throughout the body, leading to metastasis [11,15].

2.2. Enhanced Permeability and Retention (EPR) effect

Structural changes in vascular pathophysiology could provide opportunities for the use of long-circulating particulate carrier systems. The ability of vascular endothelium to present open fenestrations was described for the sinus endothelium of the liver [16], when the endothelium is perturbed by inflammatory process, hypoxic areas of infracted myocardium [17] or in tumors [18]. More particularly, tumor blood vessels are generally characterized by abnormalities such as high proportion of proliferating endothelial cells, pericyte deficiency and aberrant basement membrane formation leading to an enhanced vascular permeability. Particles, such as nanocarriers (in the size range of 20–200 nm), can extravasate and accumulate inside the interstitial space. Endothelial pores have sizes varying from 10 to 1000 nm [19]. Moreover, lymphatic vessels are absent or non-functional in tumor which contributes to inefficient drainage from the tumor tissue. Nanocarriers entered into the tumor are not removed efficiently and are thus retained in the tumor. This passive phenomenon has been called the "Enhanced Permeability and Retention (EPR) effect," discovered by Matsumura and Maeda [20–22]. The abnormal vascular architecture plays a major role for the EPR effect in tumor for selective macromolecular drug targeting at tissue level that can be summarized as follows and illustrated in Fig. 2:

(1) Extensive angiogenesis and hypervasculature
(2) Lack of smooth-muscle layer, pericytes
(3) Defective vascular architecture: fenestrations
(4) No constant blood flow and direction
(5) Inefficient lymphatic drainage that leads to enhanced retention in the interstitium of tumors
(6) Slow venous return that leads to accumulation from the interstitium of tumor

Physiological changes in blood flow within the tumors and in transport properties of tumor vessels are consequences of these vascular abnormalities. In 1987, Jain hypothesized that the osmotic pressure in tumors must be high. This high tumor interstitial fluid pressure (IFP) could be a barrier for efficient anti-cancer drug delivery [23]. It is now well known that the IFP of most solid tumors is increased. Many anti-cancer drugs — high molecular weight compounds in particular — are transported from the circulatory system through the interstitial space by convection rather than by diffusion. Increased IFP contributes to a decreased transcapillary transport in tumors, leading to a decreased uptake of drugs into tumor. In addition, IFP tends to be higher at the center of solid tumors, diminishing toward the periphery, creating a mass flow movement of fluid away from the central region of tumor. To ensure that all the tumor get an adequate drug supply, drug molecules or drug-loaded nanocarriers should migrate through the tumor interstitial space from a site of entry to remote cells. This process is hindered by high IFP. Due to their greater size, the transport of drug-loading nanocarriers is less affected by this enhanced IFP in tumors. Moreover, the microvasculature pressure in tumors is also one to two orders of magnitude higher than in normal tissues. This facilitates extravasation of nanocarriers that could otherwise have been precluded by high IFP. Many types of nanocarriers successfully overcome these barriers and selectively accumulate in the tumors [23–25].

2.3. pH

While the intracellular pH of cells within healthy tissues and tumors is similar, tumors exhibit a lower extracellular pH than normal tissues. Accordingly, although tumor pH may vary according to the tumor area, average extracellular tumor pH is between 6.0 and 7.0 whereas in normal tissues and blood, the extracellular pH of is around 7.4 [26,27]. Low pH and low pO_2 are intimately linked and a variety of insights now support their roles in the progression of tumor from in situ to invasive cancer [28]. The low extracellular tumor pH mostly arises from the high glycolysis rate in hypoxic cancer cells. Amazingly, this ATP-generating pathway is also exploited by tumor cells when oxygen is available [29]. This phenomenon named the Warburg effect emphasizes that proliferating tumor cells do not exploit the full capacity of glucose oxidation to produce energy. Both defects in the mitochondrial respiratory chain and the need of glycolysis-derived biosynthetic intermediates account for this metabolic preference (reviewed in [29]). To maintain a high glycolytic rate however requires that pyruvate is converted into lactate to generate nicotinamide adenine NAD+, a factor required by different glycolytic enzymes. Lactate itself needs to be eliminated from the cell to favor the metabolic flux and avoid cytotoxicity development. Monocarboxylate transporter will export one proton together with one lactate molecule, leading to a progressive acidification of the tumor extracellular space (and a slight alkalinisation of the cytosol). Hypoxia-induced expression of carbonic anhydrase IX (CA IX) will also contribute to exacerbate the pH gradient between the intra- and extracellular compartments through the conversion of CO_2 to bicarbonate and subsequent uptake of this weak base through the anion exchanger Cl-/bicarbonate [30].

The resulting pH gradients between intra- and extracellular tumor cells but also between the tumor mass and the host tissue are therefore potential sources of differential drug partitioning and distribution. In a low pH extracellular environment, the uncharged fraction of a weak acid increases and such a drug can thus more easily diffuse through the cell membrane. The relatively basic intracellular compartment may in turn favor the ionization of the molecule, thereby promoting the cytosolic accumulation of the drug. Alteration in this process is proposed to contribute to the multidrug resistance (MDR) phenomenon [31]. The exposure to chemotherapy may indeed favor the selection of tumor-cell clones with very acidic organelles which will trap drugs and thereby reduce their activity; if this

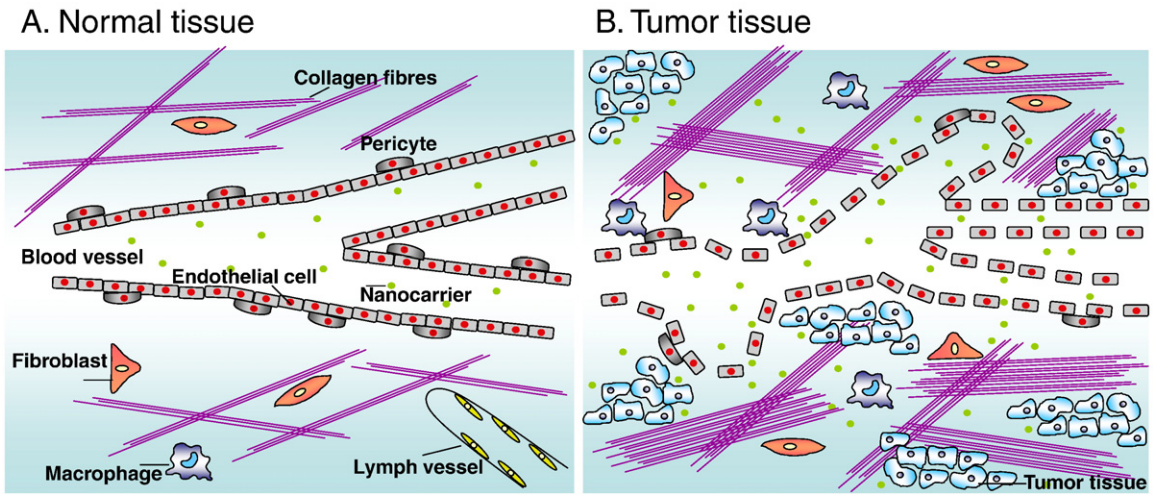

Fig. 2. Differences between normal and tumor tissues that explain the passive targeting of nanocarriers by the Enhanced Permeability and Retention effect. A. Normal tissues contain linear blood vessels maintained by pericytes. Collagen fibres, fibroblasts and macrophages are in the extracellular matrix. Lymph vessels are present. B. Tumor tissues contain defective blood vessels with many sac-like formations and fenestrations. The extracellular matrix contains more collagen fibres, fibroblasts and macrophages than in normal tissue. Lymph vessels are lacking.
Adapted from [24].

organelle is part of the secretory pathway then the drug will be transported out of the cell by exocytosis.

3. Drug targeting

3.1. Passive targeting

Passive targeting consists in the transport of nanocarriers through leaky tumor capillary fenestrations into the tumor interstitium and cells by convection or passive diffusion (Figs. 3 and 4A) [32]. The convection refers to the movement of molecules within fluids. Convection must be the predominating transport mode for most large molecules across large pores when the net filtration rate is zero. In the contrary, low-molecular weight compounds, such as oxygen, are mainly transported by diffusion, defined as a process of transport of molecules across the cell membrane, according to a gradient of concentration, and without contribution of cellular energy. Nevertheless, convection through the tumor interstitium is poor due to interstitial hypertension, leaving diffusion as the major mode of drug transport.

Selective accumulation of nanocarriers and drug then occurs by the EPR effect [32].The EPR effect is now becoming the gold standard in cancer-targeting drug designing. All nanocarriers use the EPR effect as a guiding principle. Moreover, for almost all rapidly growing solid tumors the EPR effect is applicable [22]. Indeed, EPR effect can be observed in almost all human cancers with the exception of hypovascular tumors such as prostate cancer or pancreatic cancer [21,33].

The EPR effect will be optimal if nanocarriers can evade immune surveillance and circulate for a long period. Very high local concentrations of drug-loaded nanocarriers can be achieved at the tumor site, for instance 10–50-fold higher than in normal tissue within 1–2 days [34]. To this end, at least three properties of nanocarriers are particularly important. (i) The ideal nanocarrier size should be somewhere between 10 and 100 nm. Indeed, for efficient extravasation from the fenestrations in leaky vasculature, nanocarriers should be much less than 400 nm. On the other hand, to avoid the filtration by the kidneys, nanocarriers need to be larger than 10 nm; and to avoid the a specific capture by the liver, nanocarriers need to be smaller than 100 nm. (ii) The charge of the particles should be neutral or anionic for efficient evasion of the renal elimination. (iii) The nanocarriers must be hidden from the reticulo–endothelial system, which destroys any foreign material through opsonization followed by phagocytosis [7,35].

Nevertheless, to reach passively the tumor, some limitations exist. (i) The passive targeting depends on the degree of tumor vascularization and angiogenesis. [5]. Thus extravasation of nanocarriers will

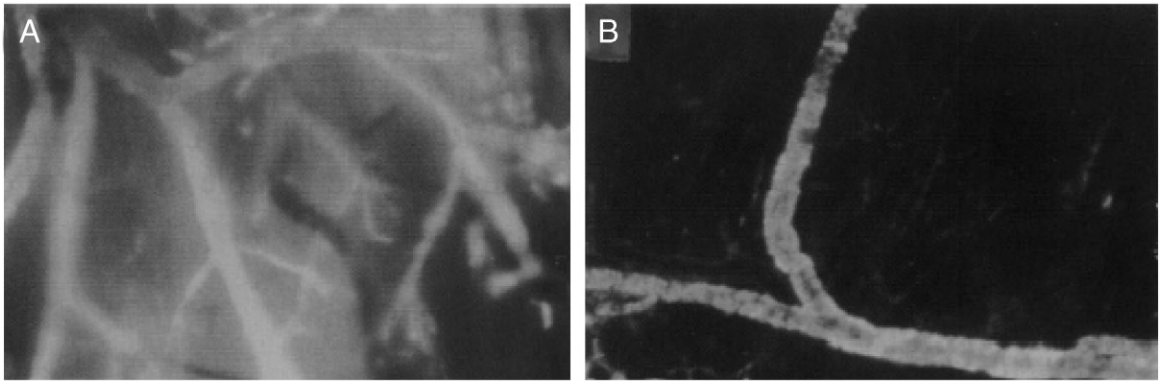

Fig. 3. Visualization of extravasation of PEG-liposomes. A. Extravasation of PEG-liposomes with 126 nm in mean diameter from tumor microvasculature was observed. Liposome localization in the tumor was perivascular. B. In normal tissue, extravasation of PEG-liposomes with 128 nm in mean diameter was not detected. Only fluorescent spots within the vessel wall were observed [33].

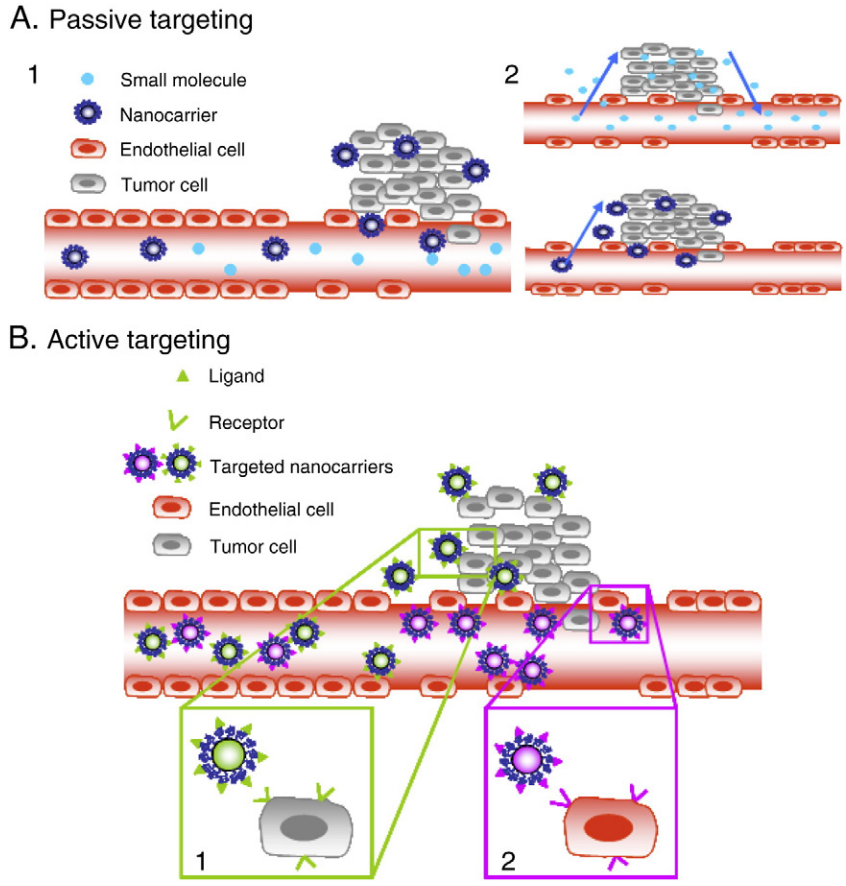

Fig. 4. A. Passive targeting of nanocarriers. (1) Nanocarriers reach tumors selectively through the leaky vasculature surrounding the tumors. (2) Schematic representation of the influence of the size for retention in the tumor tissue. Drugs alone diffuse freely in and out the tumor blood vessels because of their small size and thus their effective concentrations in the tumor decrease rapidly. By contrast, drug-loaded nanocarriers cannot diffuse back into the blood stream because of their large size, resulting in progressive accumulation: the EPR effect. B. Active targeting strategies. Ligands grafted at the surface of nanocarriers bind to receptors (over)expressed by (1) cancer cells or (2) angiogenic endothelial cells.

vary with tumor types and anatomical sites. (ii) As previously mentioned, the high interstitial fluid pressure of solid tumors avoids successful uptake and homogenous distribution of drugs in the tumor [24]. The high interstitial fluid pressure of tumors associated with the poor lymphatic drainage explain the size relationship with the EPR effect: larger and long-circulating nanocarriers (100 nm) are more retained in the tumor, whereas smaller molecules easily diffuse [36] (Fig. 4A.2).

3.2. Active targeting

In active targeting, targeting ligands are attached at the surface of the nanocarrier (Fig. 1B) for binding to appropriate receptors expressed at the target site (Fig. 4B). The ligand is chosen to bind to a receptor overexpressed by tumor cells or tumor vasculature and not expressed by normal cells. Moreover, targeted receptors should be expressed homogeneously on all targeted cells. Targeting ligands are either monoclonal antibodies (mAbs) and antibody fragments or non-antibody ligands (peptidic or not). The binding affinity of the ligands influences the tumor penetration because of the "binding-site barrier." For targets in which cells are readily accessible, typically the tumor vasculature, because of the dynamic flow environment of the bloodstream, high affinity binding appears to be preferable [37,38].

Various anti-cancer therapeutics, grouped under the name "ligand-targeted therapeutics," are divided into different classes based on the approach of drug delivery [39]. The common basic principle of all these therapeutics is the specific delivery of drugs to cancer cells. Main classes of ligand-targeted therapeutics are illustrated in Fig. 5. Antibodies (monoclonal antibody or fragments) (Fig. 5.A) target a specific receptor, interfering with signal-transduction pathways, regulating proto-oncogenes involved in cancer cells proliferation — such as trastuzumab (anti-ERBB2, **Herceptin**®), bevacizumab (anti-VEGF, **Avastin**®) or etaracizumab, a humanized anti-$\alpha_v\beta_3$ antibody (Abegrin). In this case, the active molecule plays the role of both targeting ligand and drug. Antibodies (or fragments) may only play the role of targeting ligand when they are coupled with therapeutic molecules (Fig. 5B). ^{90}yttrium–ibritumomab tiuxetan (**Zevalin**®), directed against anti-CD-20, was the first radioimmunotherapeutic received for clinical approval [40]. The first immunotoxin approved in clinical was denileukin diftitox (**Ontak**®), an interleukin (IL)-2-diphteria toxin fusion protein [41]. The only immunoconjugate to receive clinical approval is gemtuzumab ozogamicin (**Mylotarg**®) [42]. Immuno-nanocarriers (Fig. 5C) use a different approach: cytotoxic drug is encapsulated into a nanocarrier and antibodies (or fragments), the targeting ligands, are coupled to the particle surface. Finally, for targeted nanocarriers (Fig. 5D), antibodies are replaced by molecule (peptidic or not) binding to specific receptors. In this review, we focus on active targeting of immuno- and targeted nanocarriers.

In the active targeting strategy, two cellular targets can be distinguished: (i) the targeting of cancer cell (Fig. 4B.1) and (ii) the targeting of tumoral endothelium (Fig. 4B.2).

3.2.1. The targeting of cancer cell

The aim of active targeting of internalization-prone cell-surface receptors, overexpressed by cancer cells, is to improve the cellular

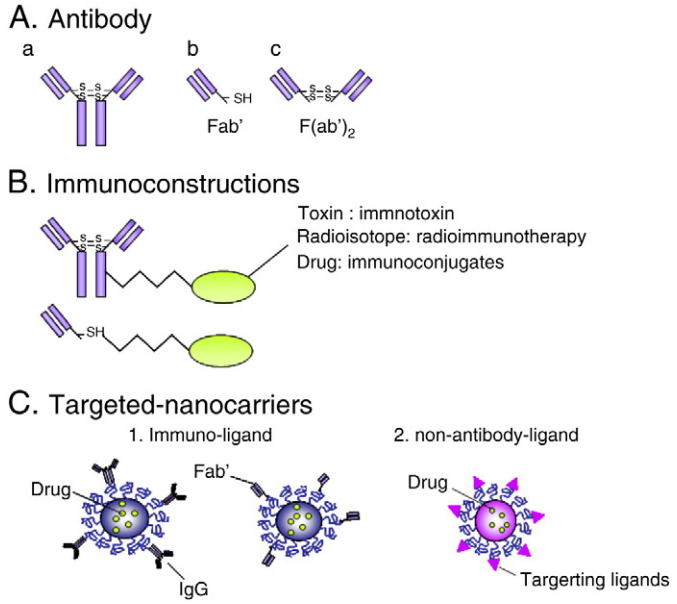

Fig. 5. Main classes of ligand-targeted therapeutics. A. Targeting antibodies are generally monoclonal immunoglobulin g (IgG) (a) or Fab′ fragments (b) or $F(ab')_2$ fragments (c). B. Immunoconstructions are formed by the linking of antibodies or fragments to therapeutic molecules. C. Targeted nanocarriers are nanocarriers presenting targeted ligands at the surface of the nanocarrier. The ligands are either monoclonal antibodies and antibody fragments (immuno-nanocarriers) or non-antibody ligands (peptidic or not). Targeted nanocarriers contain therapeutic drugs.

uptake of the nanocarriers. Thus, the active targeting is particularly attractive for the intracellular delivery of macromolecular drugs, such as DNA, siRNA and proteins. The enhanced cellular internalization rather than an increased tumor accumulation is responsible of the anti-tumoral efficacy of actively targeted nanocarriers. This is the base of the design of delivery systems targeted to endocytosis-prone surface receptors [43]. The ability of the nanocarrier to be internalized after binding to target cell is thus an important criterion in the selection of proper targeting ligands [44]. In this strategy, ligand-targeted nanocarriers will result in direct cell kill, including cytotoxicity against cells that are at the tumor periphery and are independent on the tumor vasculature [45]. The more studied internalization-prone receptors are:

(i) The transferrin receptor. Transferrin, a serum glycoprotein, transports iron through the blood and into cells by binding to the transferring receptor and subsequently being internalized via receptor-mediated endocytosis. The transferrin receptor is a vital protein involved in iron homeostasis and the regulation of cell growth. The high levels of expression of transferrin receptor in cancer cells, which may be up to 100-fold higher than the average expression of normal cells, its extracellular accessibility, its ability to internalize and its central role in the cellular pathology of human cancer, make this receptor an attractive target for cancer therapy [44,46].

(ii) The folate receptor is a well-known tumor marker that binds to the vitamin folic acid and folate–drug conjugates or folate-grafted nanocarriers with a high affinity and carries these bound molecules into the cells via receptor-mediated endocytosis. Folic acid is required in one carbon metabolic reactions and consequently, is essential for the synthesis of nucleotide bases. The alpha isoform, folate receptor-α is overexpressed on 40% of human cancers. In contrast, folate receptor-β is expressed on activated macrophages and also on the surfaces of malignant cells of hematopoietic origin [47].

(iii) Glycoproteins expressed on cell surfaces. Lectins are proteins of non-immunological origin which are able to recognize and bind to carbohydrate moieties attached to glycoproteins expressed on cell surface. Cancer cells often express different glycoproteins compared to normal cells. Lectins interaction with certain carbohydrate is very specific. Lectins can be incorporated into nanoparticles as targeting moieties that are directed to cell-surface carbohydrates (direct lectin targeting) and carbohydrates moieties can be coupled to nanoparticles to target lectins (reverse lectin targeting). The use of lectins and neoglycoconjugates for direct or reverse targeting strategies is a traditional approach of colon drug targeting [48].

(iv) The Epidermal growth factor receptor (EGFR). The EGFR is a member of the ErbB family, a family of tyrosine kinase receptors. Its activation stimulates key processes involved in tumor growth and progression, including proliferation, angiogenesis, invasion and metastasis. EGFR is frequently overexpressed in a lot of cancer, especially in breast cancer, has also been found to play a significant role in the progression of several human malignancies. Human epidermal receptor-2 (HER-2) is reported to be expressed in 14–91% of patients with breast cancer [49,50]. EGFR is expressed or overexpressed in a variety of solid tumors, including colorectal cancer, non-small-cell lung cancer and squamous cell carcinoma of the head and neck, as well as ovarian, kidney, pancreatic, and prostate cancer [51].

3.2.2. The targeting of tumoral endothelium

Destruction of the endothelium in solid tumors can result in the death of tumor cells induced by the lack of oxygen and nutrients. In 1971, Judah Folkman suggested that the tumor growth might be inhibited by preventing tumors from recruiting new blood vessels [52]. This observation is the base of the design of nanomedicines actively targeted to tumor endothelial cells [53]. By attacking the growth of the blood supply, the size and metastatic capabilities of tumors can be regulated. Thus, in this strategy, ligand-targeted nanocarriers bind to and kill angiogenic blood vessels and indirectly, the tumor cells that these vessels support, mainly in the tumor core. The advantages of the tumoral endothelium targeting are: (i) there is no need of extravasation of nanocarriers to arrive to their targeted site, (ii) the binding to their receptors is directly possible after intravenous injection, (iii) the potential risk of emerging resistance is decreased because of the genetically stability of endothelial cells as compared to tumor cells, and (iv) most of endothelial cells markers are expressed whatever the tumor type, involving an ubiquitous approach and an eventual broad application spectrum [38]. The main targets of the tumoral endothelium include:

(i) The vascular endothelial growth factors (VEGF) and their receptors, VEGFR-1 and VEGFR-2, mediate vital functions in tumor angiogenesis and neovascularization [54]. Tumor hypoxia and oncogenes upregulate VEGF levels in the tumor cells, resulting in an upregulation of VEGF receptors on tumor endothelial cells. Two major approaches to target angiogenesis via the VEGF way have been studied: (i) targeting VEGFR-2 to decrease VEGF binding and induce an endocytotic pathway and (ii) targeting VEGF to inhibit ligand binding to VEGFR-2 [1,55].

(ii) The $\alpha_v\beta_3$ integrin is an endothelial cell receptor for extracellular matrix proteins which includes fibrinogen (fibrin), vibronectin, thrombospondin, osteopontin and fibronectin [56]. The $\alpha_v\beta_3$ integrin is highly expressed on neovascular endothelial cells but poorly expressed in resting endothelial cells and most normal organs, and is important in the calcium-dependent signaling pathway leading to endothelial cell migration [1]. Cyclic or linear derivatives of RGD (Arg–Gly–Asp) oligopeptides are the most studied peptides which bind to endothelial $\alpha_v\beta_3$ integrins. The $\alpha_v\beta_3$ integrin is upregulated in both tumor cells and angiogenic endothelial cells [56].

(iii) Vascular cell adhesion molecule-1 (VCAM-1) is an immunoglobulin-like transmembrane glycoprotein that is expressed on the surface of endothelial tumor cells. VCAM-1 induces the cell-to-cell adhesion, a key step in the angiogenesis process. Overexpression of VCAM-1 is found in various cancers, including leukemia, lung and breast cancer, melanoma, renal cell carcinoma, gastric cancer and nephroblastoma [57].

(iv) The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptideases. MMPs degrade the extracellular matrix, playing an essential role in angiogenesis and metastasis more particularly in endothelial cell invasion and migration, in the formation of capillary tubes and in the recruitment of accessory cells. Membrane type 1 matrix metalloproteinase (MT1-MMP) is expressed on endothelial tumor cells, including malignancies of lung; gastric, colon and cervical carcinomas; gliomas and melanomas [58]. Aminopeptidase N/CD13, a metalloproteinase that removes amino-acids from unblocked N-terminal segments of peptides or proteins, is an endothelial cell-surface receptor involved in tumor-cell invasion, extracellular matrix degradation by tumor cells and tumor metastasis *in vitro* and *in vivo* [59]. NGR (Asn–Gly–Arg) peptide is reported to bind to the aminopeptidase [60].

Although the tumor vasculature is recognized as a major target for cancer therapies, an additional type of vascular cells, the pericytes, have been described as an alternative target that is also potentially important. It has been demonstrated that the aminopeptidase A, the membrane-associated protease, is upregulated and active in these cells [45,61].

3.3. Preclinically and clinically used tumor-targeted nanomedicines

Clinical trials of nanomedicine without targeting ligands are summarized in Table 2. The first liposomes to be approved by the regulatory authorities were **Doxil**® and **Myocet**®. Both products contain the cytotoxic drug doxorubicin. **Myocet**® is a doxorubicin formulation of uncoated liposomes whereas **Doxil**® is a PEG-liposome formulation designed to prolong blood circulation time. Free doxorubicin presents an elimination half-life time of 0.2 h. This value is enhanced to 2.5 h and 55 h for **Myocet**® and **Doxil**®, respectively [62]. **Doxil**® has been shown to induce a lower cardiotoxicity than free doxorubicin. **Myocet**® is currently used in clinical to treat breast cancer in combination with other chemotherapeutic agent (cyclophosphamide). **Doxil**® is used to treat women with metastatic breast cancer who have an increased risk of heart damage, advanced ovarian cancer and AIDS related Kaposi's sarcoma. Other liposomal systems have been approved such as **DaunoXome**® and **Onco-TCS**®, non-PEGylated liposomal formulations of daunorubicin and vincristine. Because of the well-known toxicity of Cremophor® EL, a surfactant included in the paclitaxel formulation (**Taxol**®), novel formulations of paclitaxel have been and are always intensively studied.

Abraxane®, a solvent free, albumin-bound nanoparticles of paclitaxel, also known as nab-paclitaxel, is currently used in metastatic breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. In a Phase III study, **Abraxane**® demonstrated higher response rates, a better safety profile compared with conventional paclitaxel, and improved survival in patients receiving it as second-line therapy [63]. Albumin is a plasma protein with a molecular weight of 66 kDa. Because albumin is found in the plasma of the human body, it is non-toxic and well tolerated by immune system. Albumin has attractive phamacokinetics owing to its long half-life which is particularly interesting to design a drug carrier for passive targeting. Albumin seems to help endothelial transcytosis of protein-bound and unbound plasma constituents via the binding to a cell-surface receptor (gp60) [7]. gp60 binds to caveolin-1 with subsequent formation of transcytotic vesicles (caveolae). It has been proposed that **Abraxane**® targets cancer tissues because of the high metabolic demand and active transport of plasma proteins for anabolic processes. **Abraxane**® could be transported into tumor by secreted protein acidic rich in cysteine (SPARC) or osteonectin, which binds albumin because of a sequence homology with gp60. SPARC as caveolin-1 is often expressed in some cancers (e.g. breast, lung and prostate), which could explain why albumin is known to accumulate in some tumors and thus facilitates intratumor accumulation of albumin-bound drugs [64,65].

Table 2
Examples of passively tumor-targeted nanocarriers in cancer therapy. Clinical data are extracted from http://www.clinicaltrials.gov (May 2010).

Nanocarriers	Drug	Name	Indications	Status
Polymeric micelles	Paclitaxel	**Genexol**®-PM	Breast, lung, pancreatic cancer	II–III
			Recurrent breast cancer	IV
	Doxorubicin	NK911	Various	I–II
Nanoparticles	Albumin-paclitaxel	**Abraxane**®	Metastatic breast cancer	Approved
	Doxorubicin	**Transdrug**®	Hepatocarcinoma	Approved
	Paclitaxel	**Nanoxel**®	Advanced breast cancer	I
Polymer–drug conjugates	Paclitaxel	**Xyotax**® (CT-2103)	Breast, ovarian cancer	II
			Advanced lung cancer	III
	Doxorubicin	PK1	Breast, lung, colon	II
	Paclitaxel	**Taxoprexin**®	Various	II–III
Liposomes	Doxorubicin	**Doxil**®	Ovarian, metastatic breast cancer, Kaposi sarcoma	Approved
	Doxorubicin	**Myocet**®	Breast cancer	Approved
	Daunorubicin	**DaunoXome**®	Kaposi Sarcoma	Approved
	Vincristine	**Onco-TCS**®	Non-Hodgkin lymphoma Various	Approved II
		Marqibo®	Leukemia, melanoma	II

In active targeting, the strategy consists in grafting a targeting ligand at the surface of nanocarriers to provide an enhanced selectivity and thus efficacy, as compared to the passive targeting. Although many authors report the evidence of this strategy in preclinical models, until now only three clinical trials have been conducted (Table 3): the galactosamine-targeted PHPMA doxorubicin (PK2) [66], the GAH-targeted doxorubicin-containing immunoliposomes (MCC-465) [67] and the transferrin-targeted oxaliplatin containing liposomes [68]. On the other hand, a much larger number of preclinical studies are published, using various nanomedicines and targeting ligands (Table 3).

It is important to note that for each active targeted nanocarriers described in the literature, only one strategy is exploited: the cancer cell targeting or the tumoral endothelium targeting. However, $\alpha_v\beta_3$ integrins targeted nanocarriers could be considered as double-targeting systems because $\alpha_v\beta_3$ integrins are upregulated in both tumor cells and angiogenic endothelial cells [56]. This double targeting is described for integrin antagonists as etaracizumab. In addition to its anti-angiogenic effects, etaracizumab inhibited tumor growth by directly affecting tumor cells [69]. This double targeting is not yet exploited by systems delivering chemotherapeutics. Indeed, $\alpha_v\beta_3$ integrin targeted nanocarriers were only described as systems able to target the tumor vasculature, resulting in tumor regression and inhibition of the growth of metastases [70,71].

3.4. Stimuli-sensitive nanocarriers

A new targeting strategy consists in developing “activable” or “activated” nanocarriers. Nanocarriers maintain the stealth function

Table 3
Examples of nanocarriers using the active tumoral targeting strategy.
Clinical data are extracted from http://www.clinicaltrials.gov.

Targeting ligands/*targets*	Nanocarriers	Indications/tumor cells	Status	Ref
Transferrin	Liposomes	A2780 ovarian cancer cell	In vitro	[72]
Transferrin receptor	Liposomes	C6 glioma	Preclinical	[73]
	Liposomes (MBP-426)	Metastatic solid tumor	Phase I	[68]
Folate	Liposomes	Human KB	Preclinical	[74]
Folate receptor	Nanoparticles	SKOV3	In vitro	[75]
	Micelles	Human KB	Preclinical	[76]
Lectins				
Galactosamine	Micelles	HepG2	In vitro	[77]
Asialoglyco-protein receptor	Polymer–drug conjugate (PK2)	Liver	Phase I/II	[66]
Hyaluronan	Liposomes	B16 melanoma	In vitro	[78]
CD44 receptor				
EGF				
Anti-HER-2	Liposomes	MCF-7	Preclinical	[79]
HER-2 receptor				
Anti-HER-2 MAb	Liposomes	BT-474/MCF-7 breast cancer	Preclinical	[43]
HER-2 receptor				
Anti-EGRF Mab	Liposomes	MDA-MB-468, U87 glioma	Preclinical	[80]
EGRF receptor				
VEGF				
Anti-Flk1 Mab	Nanoparticles	K1735-M2 and CT-26 tumors	Preclinical	[81]
VEGFR-2 (Flk-1)				
Anti-VEGF Mab	Magnetic nanoparticles	Human liver cancer	Preclinical	[82]
VEGF				
RGD peptide				
RGD peptide	Liposomes	B16 melanoma	Preclinical	[83]
Integrins ($\alpha_v\beta_3$)	Nanoparticles	Pancreatic/renal orthopic tumors	Preclinical	[70]
	Nanoparticles	TLT	Preclinical	[71]
	Micelles	MDA-MB-435 breast cancer	Preclinical	[84]
VCAM-1				
Anti-VCAM-1	Liposomes	Human Colo 677 tumor	Preclinical	[38]
VCAM-1				
MMP				
GPLPLR	Liposomes	Colon 26 NL-17 carcinoma	Preclinical	[85]
MT1-MMP				
Anti-MT1-MMP Fab′	Liposomes	HT1080	Preclinical	[86]
MT1-MMP				
NGR	Liposomes	Neuroblastoma	Preclinical	[87]
Aminopeptidase N				
GAH				
GAH Fab′	Liposomes (MCC-465)	Metastatic stomach cancer	Phase I	[67]

during circulation, upon arrival at the tumor site, transformation of the nanocarriers is triggered by the unique tumoral extracellular environment, allowing the drug release or the interaction with a specific target. The drug retention in nanocarriers can also be solved by application of external stimuli allowing a controlled and selective targeting of cells.

3.4.1. Internal stimuli

The issue of very acidic endosomes may be considered as a potential advantage. The use of drug-loaded micelles that destabilizes at an early endosomal pH of 6.0 should in theory maximize intracellular drug delivery and minimize drug release at the extracellular pH and at the lysosomal pH (around 5.0). An elegant study by Kim and colleagues has validated this concept [88]. These authors used a pH-sensitive doxorubicin-loaded mixed-micelles system conjugated with folic acid and documented cytotoxic effects not only in wild-type sensitive but also doxorubicin-resistant ovarian MDR cancer-cell lines. To optimize the doxorubicin release from early endosomal compartment, the pH sensitivity of the micelles was controlled by a mixture of polymers, namely polyhistidine-co-phenylalanine and poly-L-lactic acid. The concept of targeting the endosomal pH is however still in its infancy and nowadays most of the conclusive efforts to exploit differences in pH as a guide to deliver more drugs arise from the basic difference of extracellular pH in tumor versus healthy tissues. Ligands such as poly(l-histidine) or polysulfonamide actually offer systems particularly well suited to act as pH-sensitive polymeric carriers. The unsaturated nitrogen in the imidazole ring of histidine has lone pairs of electrons that endow it with pH-dependent amphoteric properties. In other words, poly (histidine) acts as a weak base that has the ability to acquire a cationic charge when the pH of the environment drops below 6.5. Incorporation of poly(histidine) ligand to polymeric micelles may thus lead to physical destabilization in the extracellular, acidic tumor medium. Lee and colleagues documented for instance that the blending of poly (L-lactic acid)/PEG block copolymer with polyHis/PEG allows to tailor the triggering pH of the polymeric micelles to acidic pH as encountered in tumors [89]. Of note, the accumulation of histidine residues inside acidic endosomal vesicles can also induce a proton sponge effect, which increases their osmolarity/swelling and favors payload delivery.

Similarly, exposure to the acidic extracellular tumor pH leads to the neutralization of polysulfonamides (which are negatively charged

at pH 7.4). Consecutive disruption of ionic interactions may then facilitate the delivery of the nanoparticle content or even expose so far hidden motifs to further facilitate delivery. This may be particularly suited to allow the exposure of cell-penetrating peptides endowed with the capacity to translocate their conjugated moieties into the cell [90]. Such a shielding/deshielding mechanism was recently reported to expose cationic TAT (HIV transactivator of transcription) peptide in the tumor microenvironment [91]. Tumor extracellular/endosomal pH-sensitive nanocarriers have therefore the potential to overcome the lack of selectivity of conventional anti-cancer modalities and together with EPR to increase the efficacy of therapeutic entities including siRNA, drugs or radioisotopes.

Others targeted therapies able to specifically kill tumor cells are based on tumor site-specific enzymatically activation of prodrugs. Either a selected enzyme is accumulated in the tumor by guiding the enzyme to the neoplasic cells or a harmless prodrug is applied and specifically converted by this enzyme into a cytotoxic drug only at the tumor site [92]. This targeted enzyme prodrug strategy has no application, to our knowledge, to nanocarriers. Nevertheless, nanocarriers may also be activated by enzyme overexpressed in the tumor. Indeed, specific enzymes of tumor may control the release of a drug from a nanocarrier by the cleavage of a linker of the polymers [93]. For example, a drug–polymer conjugate was created by conjugating methotrexate to dextran via a peptide linker that could be cleaved by MMP-2and MMP-9, 2 important tumor-associated enzymes [35].

Redox/thiol sensitive polymers are another class of responsive polymers. The conversion of disulfides and thiols is a key step in many biological processes. Disulfide bonds can be converted reversibly to thiols by various reducing agents and undergo disulfide exchange in the presence of other thiols. For this reason, polymers containing disulfide linkages can be considered both redox and thiol-responsive [93]. For example, it was demonstrated that supromolecular polymer surfactant complexes can form micelles susceptible to thiol-induced dissociation [94].

3.4.2. External stimuli

Many pathological areas show distinct hyperthermia (e.g. 42 °C in human ovarian carcinoma). Temperature-sensitive nanocarriers contain a polymer with a low critical solution temperature (LCST). Because of the precipitation of the polymer when the temperature is above the LCST (in the tumor), the nanocarrier structure is damaged and the drug is released. The poly(N-isopropylacrylamide) (NIPAM) is the most widely used polymers for thermoresponsive nanocarriers [95]. Local heating of the tumor may be achieved by various physical means, among which the least invasive, easiest and cheapest is ultrasound.

Magnetic nanoparticles were firstly designed for magnetic resonance imaging via passive targeting. Currently, some superparamagnetic iron oxide nanoparticles (SPION) are in early clinical trials and several formulations have been approved for medical imaging, such as Feridex®, Combidex® or Ferumoxytol® [96,97]. Magnetic nanoparticles are also studied for drug targeting applications. A number of SPION systems have been coated with targeting ligands (active targeting). Under the influence of external magnets, it is possible to guide nanoparticles to a particular targeted site [98,99]. The increasing local temperature obtained by using SPION in an alternating magnetic field allowed the elimination of the tumor. This principle is called the “magnetic thermal ablation” [100].

Photo-responsive polymers are molecules that change their properties when they are irradiated with light of the appropriate wavelength. Typically, the light induces structural transformations of specific functions of this kind of polymers. For example, when exposed to UV irradiation, cleavage of the pyrenyl-methyl esters caused the pyrene-containing hydrophobic methacrylate units to be transformed to hydrophilic methacrylic acid units, resulting in micelle dissociation [94]. Because light of UV and visible wavelengths is readily absorbed by the skin, these systems may present some limitations. For this reason, other polymers sensitive to infra-red or near infra-red lights are studied [101,102].

The drug delivery and the release from nanocarriers may also be triggered by external ultrasound. Ultrasonic waves can be used to induce either thermal or mechanical effects. Local heating can be induced using high-intensity focused ultrasound (HIFU), inducing phase transition of the polymers, which involves the drug release from nanocarriers. The drug release may also be induced by mechanical effects associated with ultrasound, such as transient cavitation [103].

3.5. Multifunctional nanocarriers

Different drug delivery systems are currently studied. These systems are typically based on only one useful function(s): (i) the longevity (PEGylation), (ii) the targetability (passive or active targeting) and (iii) the stimuli sensitivity (pH, temperature, ultrasound, etc.). An increasing number of publications deal with the combination of these functions for the designing of drug delivery systems [104]. Multifunctional nanocarriers may combine therapeutic and diagnostic strategies but also different therapeutic approaches. Therefore, multifunctional drug carriers may combine the targetability and the stimuli sensitivity. Cyclic NGR peptide targeted thermally sensitive liposome was designed for binding preferentially to CD13/aminopeptidase N overexpressed in tumor vasculature; these liposomes released doxorubicin at 41 °C (in the tumor) while the release was minimal at 37 °C. This system allows the improvement of the drug release and the total accumulation in the tumor [105]. Biotin targeted thermoresponsive micelles were also described [106]. Another example is the double-targeted pH-responsive nanocarriers. Liposomes and micelles were protected by low pH-cleavable PEG chains. Monoclonal antibodies were attached at the surface of non-cleavable longer PEG chains and additional functions (TAT peptide) were attached at the surface of non-cleavable anchor shorter than the cleavable PEG chain. In addition to prolonged circulation (PEGylation), this system combined the target recognition (antibody) and the activation of the system under local stimuli (lowered pH) (Fig. 6) [107]. Magnetic nanoparticles were conjugated with anti-VEGF monoclonal antibody as double-targeting vector for the radioimmunotherapy of liver cancer with an external magnetic field [106].

It is also possible to design drug delivery systems by combining two forms of targetability: the cancer cell targeting and the tumoral endothelium targeting. Doxorubicin was formulated in liposomes targeted against tumor cells via anti-G2 monoclonal antibodies and against the tumor vasculature via the NGR peptide which targets the aminopeptidase N (CD13). It was demonstrated that these two therapies are complementary. Circulating tumor cells, very early metastases and the dividing rim of mature tumors will be most sensitive to tumor-targeted therapies, whereas angiogenic metastases and more mature tumors will be most sensitive to antivascular therapies [45]. The combination of different strategies results in multifunctional nanocarriers of new generation. They represent a growing area of drug delivery system research (Fig. 6).

4. Conclusions and perspectives

In conclusion, we have described the tumor microenvironment to better understand the possibilities and the opportunities to design new passive or active targeted drug delivery systems. Nanocarriers can escape from tumor vasculature through the leaky endothelial tissue that surrounds the tumor and then accumulate in certain solid tumors by the EPR effect. This phenomenon is called the “*passive targeting*.” The basis for increased tumor specificity is the differential accumulation of drug-loaded nanocarriers in tumor tissue versus normal tissue [108,109]. Target ligands attached to the surface of

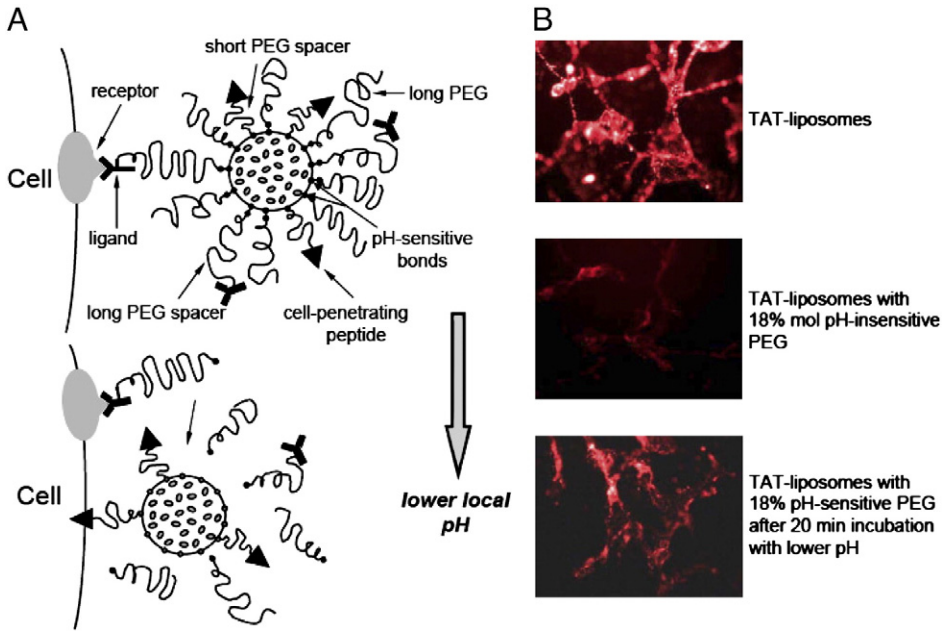

Fig. 6. Double-targeted «smart» nanocarrier with temporarily "hidden" function. A. Schematic representation. Polymeric chains are attached to the carrier via low pH-degradable bonds. After accumulation in the tumor due to PEG (longevity) and ligand (specific targeting), local pH-dependent removal of protecting PEG chains allows the direct interaction of the cell-penetrating functions of the carrier with the tumor cells. B. Fluorescence microscopy showing interaction of "smart" TAT peptide-modified liposomes. Rhodamine-labeled TAT-liposomes are effectively taken by cells. The attachment of PEG chains to the liposomes surface (18%) leads to the almost completely blocked uptake of TAT-mediated liposomes. However, if PEG is attached via pH-sensitive bonds, PEG chains are eliminated from the liposome surface and thus the TAT-mediated uptake of the liposomes by cells is good [104,107].

nanocarriers may act as "homing devices," improving the selective delivery of drug to specific tissue and cells. Among various ligands currently developed allowing the "*active targeting*" of tumors some target tumor endothelial cells while others targets cancer cells themselves [110].

The US National Science Foundation estimates that the nanotechnology market will be worth $ 1 trillion by 2015. The National Cancer Institute is engaged in efforts to harness the power of nanotechnology to radically change the way we diagnose, image and treat cancer. This includes the design of targeted contrast agents that improve the resolution of tumor imaging and nanomedicines able to act on specific cells. Nanomedicines as drug delivery systems are expected to change the pharmaceutical landscape in the future, offering new opportunities to market drugs that until now could not be administered (poorly-water soluble drugs, bioactive macromolecules). Fewer clinical examples illustrate that nanotechnology has enabled the existence of new therapeutics that would otherwise not exist. The benefit of a targeted drug delivery system over the equivalent non-targeted system is expected to be substantial. The attractive properties of nanomedicines include their ability of controlled release of drugs, the targeting of specific tissues and the biocompatibility. Nanomedicines offer the possibility to modify the pharmacokinetics parameters and to decrease the systemic toxicity of drugs. In the current context where it is required to find not only more effective but also less toxic treatments, nanomedicines find their place. Indeed, in a global approach of treatments, today, the efficiency but also the patient's quality of life are taken into account. Regarding the growing number of clinical trials of nanomedicines combined with radiotherapy or with conventional chemotherapy, there is an evidence of the success of these therapies in the future [111].

According to the current clinical trials, combinations of treatments seem to be the future. Indeed, before a new drug delivery system is approved, it is already being tested in combination with other treatments. Drug delivery systems may thus be associated with vasoactive agents to increase the EPR-mediated targeting [109], with radiation or with conventional chemotherapy. It is also possible to associate drug delivery systems with different targeting strategies. Therefore, there is no doubt that nanocarriers, particularly multifunctional systems or associations, will exist as main therapeutic arsenal in the future. Nevertheless until now, some limitations exist: (i) the major limitation impeding the entry of targeted nanomedicines onto the market is that innovative research ideas within academia are not exploited in collaboration with the pharmaceutical industry [53]. (ii) A new subdiscipline of nanotechnology called "nanotoxicology" has emerged. Indeed, *in vivo* systems are extremely complex and the interactions of nanocarriers with biological components are vast. As expected, the size and surface properties of nanocarriers modify the behavior of these components in the body. More data are needed to understand their structure–property relationship. Some nanomedicines received regulatory approvals showing their biocompatibility while others were not tested. Studies and regulations are necessary in order to fully define the biocompatibility of nanocarriers in humans. (iii) Clinical trials of combined treatments are difficult to conduct particularly because the proof of principle and complete toxicology data are difficult to establish.

In the future, we can expect the emergence of many nanotechnology platforms for drug delivery applications. Nanotechnology will change the very foundations of cancer diagnosis, treatment and prevention.

References

[1] J.D. Byrne, T. Betancourt, L. Brannon-Peppas, Active targeting schemes for nanoparticle systems in cancer therapeutics, Adv. Drug Deliv. Rev. 60 (2008) 1615–1626.
[2] J.H. Park, S. Lee, J.H. Kim, K. Park, K. Kim, I.C. Kwon, Polymeric nanomedecine for cancer therapy, Prog. Polym. Sci. 33 (2008) 113–137.
[3] S.M. Moghimi, A.C. Hunter, J.C. Murray, Nanomedicine: current status and future prospects, FASEB J. 19 (2005) 311–330.
[4] P. Ehrlich, The Collected Papers of Paul Ehrlich, Pergamon, London, 1960, p. 3.
[5] Y.H. Bae, Drug targeting and tumor heterogeneity, J. Control. Release 133 (2009) 2–3.
[6] H. Hillaireau, P. Couvreur, Nanocarriers' entry into the cell: relevance to drug delivery, Cell. Mol. Life Sci. 66 (2009) 2873–2896.

[7] Y. Malam, M. Loizidou, A.M. Seifalian, Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer, Trends Pharmacol. Sci. 30 (2009) 592–599.
[8] B.K. Nanjwade, H.M. Bechra, G.K. Derkar, F.V. Manvi, V.K. Nanjwade, Dendrimers: emerging polymers for drug-delivery systems, Eur. J. Pharm. Sci. 38 (2009) 185–196.
[9] R. Duncan, Polymer conjugates as anticancer nanomedicines, Nat. Rev. Cancer 6 (2006) 688–701.
[10] D.E. Owens III, N.A. Peppas, Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles, Int. J. Pharm. 307 (2006) 93–102.
[11] G. Bergers, L.E. Benjamin, Tumorigenesis and the angiogenic switch, Nat. Rev. Cancer 3 (2003) 401–410.
[12] P. Carmeliet, Mechanisms of angiogenesis and arteriogenesis, Nat. Med. 6 (2000) 389–395.
[13] C.J. Avraamides, B. Garmy-Susini, J.A. Varner, Integrins in angiogenesis and lymphangiogenesis, Nat. Rev. Cancer 8 (2008) 604–617.
[14] T.H. Stollman, T.J. Ruers, W.J. Oyen, O.C. Boerman, New targeted probes for radioimaging of angiogenesis, Methods 48 (2009) 188–192.
[15] G.N. Naumov, L.A. Akslen, J. Folkman, Role of angiogenesis in human tumor dormancy: animal models of the angiogenic switch, Cell Cycle 5 (2006) 1779–1787.
[16] F.H. Roerdink, J. Dijkstra, H.H. Spanjer, G.L. Scherphof, Interaction of liposomes with hepatocytes and Kupffer cells in vivo and in vitro, Biochem. Soc. Trans. 12 (1984) 335–336.
[17] T.N. Palmer, V.J. Caride, M.A. Caldecourt, J. Twickler, V. Abdullah, The mechanism of liposome accumulation in infarction, Biochim. Biophys. Acta 797 (1984) 363–368.
[18] R.K. Jain, Delivery of novel therapeutic agents in tumors: physiological barriers and strategies, J. Natl Cancer Inst. 81 (1989) 570–576.
[19] V.P. Torchilin, Drug targeting, Eur. J. Pharm. Sci. 11 (Suppl 2) (2000) S81–S91.
[20] Y. Matsumura, H. Maeda, A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs, Cancer Res. 46 (1986) 6387–6392.
[21] H. Maeda, T. Sawa, T. Konno, Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS, J. Control. Release 74 (2001) 47–61.
[22] H. Maeda, G.Y. Bharate, J. Daruwalla, Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect, Eur. J. Pharm. Biopharm. 71 (2009) 409–419.
[23] R.K. Jain, Transport of molecules in the tumor interstitium: a review, Cancer Res. 47 (1987) 3039–3051.
[24] C.H. Heldin, K. Rubin, K. Pietras, A. Ostman, High interstitial fluid pressure — an obstacle in cancer therapy, Nat. Rev. Cancer 4 (2004) 806–813.
[25] C. Bouzin, O. Feron, Targeting tumor stroma and exploiting mature tumor vasculature to improve anti-cancer drug delivery, Drug Resist. Updat. 10 (2007) 109–120.
[26] R. van Sluis, Z.M. Bhujwalla, N. Raghunand, P. Ballesteros, J. Alvarez, S. Cerdan, J.P. Galons, R.J. Gillies, In vivo imaging of extracellular pH using 1H MRSI, Magn. Reson. Med. 41 (1999) 743–750.
[27] R.A. Cardone, V. Casavola, S.J. Reshkin, The role of disturbed pH dynamics and the Na+/H+ exchanger in metastasis, Nat. Rev. Cancer 5 (2005) 786–795.
[28] J.S. Fang, R.D. Gillies, R.A. Gatenby, Adaptation to hypoxia and acidosis in carcinogenesis and tumor progression, Semin. Cancer Biol. 18 (2008) 330–337.
[29] O. Feron, Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells, Radiother. Oncol. 92 (2009) 329–333.
[30] M.C. Brahimi-Horn, J. Pouyssegur, Oxygen, a source of life and stress, FEBS Lett. 581 (2007) 3582–3591.
[31] S.M. Simon, Role of organelle pH in tumor cell biology and drug resistance, Drug Discov. Today 4 (1999) 32–38.
[32] B. Haley, E. Frenkel, Nanoparticles for drug delivery in cancer treatment, Urol. Oncol. 26 (2008) 57–64.
[33] S. Unezaki, K. Maruyama, J. Hosoda, I. Nagae, Y. Koyanagi, M. Nakata, O. Ishida, M. Iwatsuru, S. Tsuchiya, Direct measurement of extravasation of polyethyleneglycol-coated liposomes into solid tumor tissue by in vivo fluorescence, Int. J. Pharm. 144 (1996) 11–17.
[34] A.K. Iyer, G. Khaled, J. Fang, H. Maeda, Exploiting the enhanced permeability and retention effect for tumor targeting, Drug Discov. Today 11 (2006) 812–818.
[35] E. Gullotti, Y. Yeo, Extracellularly activated nanocarriers: a new paradigm of tumor targeted drug delivery, Mol. Pharm. 6 (2009) 1041–1051.
[36] K.F. Pirollo, E.H. Chang, Does a targeting ligand influence nanoparticle tumor localization or uptake? Trends Biotechnol. 26 (2008) 552–558.
[37] G.P. Adams, R. Schier, A.M. McCall, H.H. Simmons, E.M. Horak, R.K. Alpaugh, J.D. Marks, L.M. Weiner, High affinity restricts the localization and tumor penetration of single-chain fv antibody molecules, Cancer Res. 61 (2001) 4750–4755.
[38] S. Gosk, T. Moos, C. Gottstein, G. Bendas, VCAM-1 directed immunoliposomes selectively target tumor vasculature in vivo, Biochim. Biophys. Acta 1778 (2008) 854–863.
[39] T.M. Allen, Ligand-targeted therapeutics in anticancer therapy, Nat. Rev. Cancer 2 (2002) 750–763.
[40] G.A. Wiseman, C.A. White, R.B. Sparks, W.D. Erwin, D.A. Podoloff, D. Lamonica, N.L. Bartlett, J.A. Parker, W.L. Dunn, S.M. Spies, R. Belanger, T.E. Witzig, B.R. Leigh, Biodistribution and dosimetry results from a phase III prospectively randomized controlled trial of Zevalin radioimmunotherapy for low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma, Crit. Rev. Oncol. Hematol. 39 (2001) 181–194.
[41] M. Duvic, T.M. Kuzel, E.A. Olsen, A.G. Martin, F.M. Foss, Y.H. Kim, P.W. Heald, P. Bacha, J. Nichols, A. Liepa, Quality-of-life improvements in cutaneous T-cell lymphoma patients treated with denileukin diftitox (ONTAK), Clin. Lymphoma 2 (2002) 222–228.
[42] J.G. Jurcic, Antibody therapy for residual disease in acute myelogenous leukemia, Crit. Rev. Oncol. Hematol. 38 (2001) 37–45.
[43] 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.
[44] K. Cho, X. Wang, S. Nie, Z.G. Chen, D.M. Shin, Therapeutic nanoparticles for drug delivery in cancer, Clin. Cancer Res. 14 (2008) 1310–1316.
[45] F. Pastorino, C. Brignole, P.D. Di, B. Nico, A. Pezzolo, D. Marimpietri, G. Pagnan, F. Piccardi, M. Cilli, R. Longhi, D. Ribatti, A. Corti, T.M. Allen, M. Ponzoni, Targeting liposomal chemotherapy via both tumor cell-specific and tumor vasculature-specific ligands potentiates therapeutic efficacy, Cancer Res. 66 (2006) 10073–10082.
[46] T.R. Daniels, T. Delgado, G. Helguera, M.L. Penichet, The transferrin receptor part II: targeted delivery of therapeutic agents into cancer cells, Clin. Immunol. 121 (2006) 159–176.
[47] P.S. Low, S.A. Kularatne, Folate-targeted therapeutic and imaging agents for cancer, Curr. Opin. Chem. Biol. 13 (2009) 256–262.
[48] T. Minko, Drug targeting to the colon with lectins and neoglycoconjugates, Adv. Drug Deliv. Rev. 56 (2004) 491–509.
[49] S. Acharya, F. Dilnawaz, S.K. Sahoo, Targeted epidermal growth factor receptor nanoparticle bioconjugates for breast cancer therapy, Biomaterials 30 (2009) 5737–5750.
[50] M. Scaltriti, J. Baselga, The epidermal growth factor receptor pathway: a model for targeted therapy, Clin. Cancer Res. 12 (2006) 5268–5272.
[51] G. Lurje, H.J. Lenz, EGFR signaling and drug discovery, Oncology 77 (2009) 400–410.
[52] J. Folkman, Transplacental carcinogenesis by stilbestrol, N Engl J. Med. 285 (1971) 404–405.
[53] T. Lammers, W.E. Hennink, G. Storm, Tumour-targeted nanomedicines: principles and practice, Br. J. Cancer 99 (2008) 392–397.
[54] M. Shadidi, M. Sioud, Selective targeting of cancer cells using synthetic peptides, Drug Resist. Updat. 6 (2003) 363–371.
[55] P. Carmeliet, VEGF as a key mediator of angiogenesis in cancer, Oncology 69 (Suppl 3) (2005) 4–10.
[56] J.S. Desgrosellier, D.A. Cheresh, Integrins in cancer: biological implications and therapeutic opportunities, Nat. Rev. Cancer 10 (2010) 9–22.
[57] A. Dienst, A. Grunow, M. Unruh, B. Rabausch, J.E. Nor, J.W. Fries, C. Gottstein, Specific occlusion of murine and human tumor vasculature by VCAM-1-targeted recombinant fusion proteins, J. Natl Cancer Inst. 97 (2005) 733–747.
[58] L. Genis, B.G. Galvez, P. Gonzalo, A.G. Arroyo, MT1-MMP: universal or particular player in angiogenesis? Cancer Metastasis Rev. 25 (2006) 77–86.
[59] I. Saiki, H. Fujii, J. Yoneda, F. Abe, M. Nakajima, T. Tsuruo, I. Azuma, Role of aminopeptidase N (CD13) in tumor-cell invasion and extracellular matrix degradation, Int. J. Cancer 54 (1993) 137–143.
[60] R. Pasqualini, E. Koivunen, R. Kain, J. Lahdenranta, M. Sakamoto, A. Stryhn, R.A. Ashmun, L.H. Shapiro, W. Arap, E. Ruoslahti, Aminopeptidase N is a receptor for tumor-homing peptides and a target for inhibiting angiogenesis, Cancer Res. 60 (2000) 722–727.
[61] R.O. Schlingemann, E. Oosterwijk, P. Wesseling, F.J. Rietveld, D.J. Ruiter, Aminopeptidase a is a constituent of activated pericytes in angiogenesis, J. Pathol. 179 (1996) 436–442.
[62] R.D. Hofheinz, S.U. Gnad-Vogt, U. Beyer, A. Hochhaus, Liposomal encapsulated anti-cancer drugs, Anticancer Drugs 16 (2005) 691–707.
[63] W.J. Gradishar, S. Tjulandin, N. Davidson, H. Shaw, N. Desai, P. Bhar, M. Hawkins, J. O'Shaughnessy, Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer, J. Clin. Oncol. 23 (2005) 7794–7803.
[64] N. Desai, Increased antitumor activity, intratumor paclitaxel concentrations and endothelial cell transport of Cremophor-free, albumin-bound paclitaxel, ABI-007, compared with Cremophor-based paclitaxel, Clin. Cancer Res. 12 (2006) 1317–1324.
[65] E. Miele, G. Spinelli, E. Miele, F. Tomao, S. Tomao, Albumin-bound formulation of paclitaxel (Abraxane ABI-007) in the treatment of breast cancer, Int. J. Nanomedicine 4 (2009) 99–105.
[66] L.W. Seymour, D.R. Ferry, D. Anderson, S. Hesslewood, P.J. Julyan, R. Poyner, J. Doran, A.M. Young, S. Burtles, D.J. Kerr, Hepatic drug targeting: phase I evaluation of polymer-bound doxorubicin, J. Clin. Oncol. 20 (2002) 1668–1676.
[67] 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.
[68] 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.
[69] K. Mulgrew, K. Kinneer, X.T. Yao, B.K. Ward, M.M. Damschroder, B. Walsh, S.Y. Mao, C. Gao, P.A. Kiener, S. Coats, M.S. Kinch, D.A. Tice, Direct targeting of alphavbeta3 integrin on tumor cells with a monoclonal antibody, Abegrin, Mol. Cancer Ther. 5 (2006) 3122–3129.
[70] E.A. Murphy, B.K. Majeti, L.A. Barnes, M. Makale, S.M. Weis, K. Lutu-Fuga, W. Wrasidlo, D.A. Cheresh, Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis, Proc. Natl Acad. Sci. USA 105 (2008) 9343–9348.

[71] F. Danhier, B. Vroman, N. Lecouturier, N. Crokart, V. Pourcelle, H. Freichels, C. Jérôme, J. Marchand-Brynaert, O. Feron, V. Préat, Targeting of tumor endothelium by RGD-grafted PLGA-nanoparticles loaded with Paclitaxel, J. Control. Release 140 (2009) 166–173.
[72] M.L. Krieger, N. Eckstein, V. Schneider, M. Koch, H.D. Royer, U. Jaehde, G. Bendas, Overcoming cisplatin resistance of ovarian cancer cells by targeted liposomes in vitro, Int. J. Pharm. 389 (2010) 10–17.
[73] X. Ying, H. Wen, W.L. Lu, J. Dua, J. Guo, W. Tian, Y. Men, Y. Zhang, R.J. Li, T.Y. Yang, D.W. Hang, J.N. Lou, L.R. Zhang, Q. Zhang, Dual-targeting daunorubicin liposomes improve the therapeutic efficacy of brain glioma in animals, J. Control. Release 141 (2010) 183–192.
[74] A. Gabizon, A.T. Horowitz, D. Goren, D. Tzemach, H. Shmeeda, S. Zalipsky, In vivo fate of folate-targeted polyethylene-glycol liposomes in tumor-bearing mice, Clin. Cancer Res. 9 (2003) 6551–6559.
[75] F. Esmaeili, M.H. Ghahremani, S.N. Ostad, F. Atyabi, M. Seyedabadi, M.R. Malekshahi, M. Amini, R. Dinarvand, Folate-receptor-targeted delivery of docetaxel nanoparticles prepared by PLGA-PEG-folate conjugate, J. Drug Target. 16 (2008) 415–423.
[76] H.S. Yoo, T.G. Park, Folate receptor targeted biodegradable polymeric doxorubicin micelles, J. Control. Release 96 (2004) 273–283.
[77] Y.C. Wang, X.Q. Liu, T.M. Sun, M.H. Xiong, J. Wang, Functionalized micelles from block copolymer of polyphosphoester and poly(epsilon-caprolactone) for receptor-mediated drug delivery, J. Control. Release 128 (2008) 32–40.
[78] R.E. Eliaz, S. Nir, C. Marty, F.C. Szoka Jr., Determination and modeling of kinetics of cancer cell killing by doxorubicin and doxorubicin encapsulated in targeted liposomes, Cancer Res. 64 (2004) 711–718.
[79] J.W. Park, K. Hong, D.B. Kirpotin, G. Colbern, R. Shalaby, J. Baselga, Y. Shao, U.B. Nielsen, J.D. Marks, D. Moore, D. Papahadjopoulos, C.C. Benz, Anti-HER2 immunoliposomes: enhanced efficacy attributable to targeted delivery, Clin. Cancer Res. 8 (2002) 1172–1181.
[80] C. Mamot, D.C. Drummond, C.O. Noble, V. Kallab, Z. Guo, K. Hong, D.B. Kirpotin, J.W. Park, Epidermal growth factor receptor-targeted immunoliposomes significantly enhance the efficacy of multiple anticancer drugs in vivo, Cancer Res. 65 (2005) 11631–11638.
[81] L. Li, C.A. Wartchow, S.N. Danthi, Z. Shen, N. Dechene, J. Pease, H.S. Choi, T. Doede, P. Chu, S. Ning, D.Y. Lee, M.D. Bednarski, S.J. Knox, A novel antiangiogenesis therapy using an integrin antagonist or anti-Flk-1 antibody coated 90Y-labeled nanoparticles, Int. J. Radiat. Oncol. Biol. Phys. 58 (2004) 1215–1227.
[82] C. Cheng, H. Wei, J.L. Zhu, C. Chang, H. Cheng, C. Li, S.X. Cheng, X.Z. Zhang, R.X. Zhuo, Functionalized thermoresponsive micelles self-assembled from biotin-PEG-b-P(NIPAAm-co-HMAAm)-b-PMMA for tumor cell target, Bioconjug. Chem. 19 (2008) 1194–1201.
[83] X.B. Xiong, Y. Huang, W.L. Lu, X. Zhang, H. Zhang, T. Nagai, Q. Zhang, Intracellular delivery of doxorubicin with RGD-modified sterically stabilized liposomes for an improved antitumor efficacy: in vitro and in vivo, J. Pharm. Sci. 94 (2005) 1782–1793.
[84] Z. Hu, F. Luo, Y. Pan, C. Hou, L. Ren, J. Chen, J. Wang, Y. Zhang, Arg–Gly–Asp (RGD) peptide conjugated poly(lactic acid)-poly(ethylene oxide) micelle for targeted drug delivery, J. Biomed. Mater. Res. A 85 (2008) 797–807.
[85] M. Kondo, T. Asai, Y. Katanasaka, Y. Sadzuka, H. Tsukada, K. Ogino, T. Taki, K. Baba, N. Oku, Anti-neovascular therapy by liposomal drug targeted to membrane type-1 matrix metalloproteinase, Int. J. Cancer 108 (2004) 301–306.
[86] H. Hatakeyama, H. Akita, E. Ishida, K. Hashimoto, H. Kobayashi, T. Aoki, J. Yasuda, K. Obata, H. Kikuchi, T. Ishida, H. Kiwada, H. Harashima, Tumor targeting of doxorubicin by anti-MT1-MMP antibody-modified PEG liposomes, Int. J. Pharm. 342 (2007) 194–200.
[87] F. Pastorino, C. Brignole, D. Marimpietri, M. Cilli, C. Gambini, D. Ribatti, R. Longhi, T.M. Allen, A. Corti, M. Ponzoni, Vascular damage and anti-angiogenic effects of tumor vessel-targeted liposomal chemotherapy, Cancer Res. 63 (2003) 7400–7409.
[88] D. Kim, E.S. Lee, K.T. Oh, Z.G. Gao, Y.H. Bae, Doxorubicin-loaded polymeric micelle overcomes multidrug resistance of cancer by double-targeting folate receptor and early endosomal pH, Small 4 (2008) 2043–2050.
[89] E.S. Lee, K. Na, Y.H. Bae, Polymeric micelle for tumor pH and folate-mediated targeting, J. Control. Release 91 (2003) 103–113.
[90] C.C. Berry, Intracellular delivery of nanoparticles via the HIV-1 tat peptide, Nanomed. (Lond.) 3 (2008) 357–365.
[91] 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.
[92] N. Schellmann, M. Deckert, D. Bachran, H. Fuchs, C. Bachran, Targeted enzyme prodrug therapies, Mini Rev. Med. Chem. 10 (2010) 887–904.
[93] D. Roy, J. Cambre, B. Sumerlin, Future perspectives and recent advances in stimuli-responsive materials, Prog. Polym. Sci. 35 (2010) 278–301.
[94] S. Ghosh, V. Yesilyurt, E. Savariar, K. Irvin, S. Thayumanavan, Redox, ionic strength, and pH sensitive supramolecular polymer assemblies, J. Polym. Sci. Part A: Polym. Chem. 47 (2009) 1052–1060.
[95] K. Kono, K. Yoshino, T. Takagishi, Effect of poly(ethylene glycol) grafts on temperature-sensitivity of thermosensitive polymer-modified liposomes, J. Control. Release 80 (2002) 321–332.
[96] O. Veiseh, J.W. Gunn, M. Zhang, Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging, Adv. Drug Deliv. Rev. 63 (2010) 284–304.
[97] M. Mahoudi, S. Sant, B. Wang, S. Laurent, T. Sen, Superparamagnetic iron oxide nanoparticles (SPIONs): development, surface modification and applications in chemotherapy Adv. Drug Deliv. Rev. (in press), doi:10.1016/j.addr.2010.05.006.
[98] S.C. McBain, H.H. Yiu, J. Dobson, Magnetic nanoparticles for gene and drug delivery, Int. J. Nanomedicine 3 (2008) 169–180.
[99] M.W. Wilson, R.K. Kerlan Jr., N.A. Fidelman, A.P. Venook, J.M. LaBerge, J. Koda, R.L. Gordon, Hepatocellular carcinoma: regional therapy with a magnetic targeted carrier bound to doxorubicin in a dual MR imaging/ conventional angiography suite—initial experience with four patients, Radiology 230 (2004) 287–293.
[100] I. Hilger, R. Hiergeist, R. Hergt, K. Winnefeld, H. Schubert, W.A. Kaiser, Thermal ablation of tumors using magnetic nanoparticles: an in vivo feasibility study, Invest Radiol. 37 (2002) 580–586.
[101] A. Goodwin, J. Mynar, Y. Ma, G. Fleming, J. Frechet, Synthetic micelle sensitive to IR light via a two-photon process, J. Am. Chem. Soc. 127 (2005) 9952–9953.
[102] J. Jiang, X. Tong, D. Morris, Y. Zhao, Toward photocontrolled release using light-dissociable block copolymer micelles, Macromolecules 39 (2006) 4633–4640.
[103] A. Schroeder, J. Kost, Y. Barenholz, Ultrasound, liposomes, and drug delivery: principles for using ultrasound to control the release of drugs from liposomes, Chem. Phys. Lipids 162 (2009) 1–16.
[104] V. Torchilin, Multifunctional and stimuli-sensitive pharmaceutical nanocarriers, Eur. J. Pharm. Biopharm. 71 (2009) 431–444.
[105] A.H. Negussie, J.L. Miller, G. Reddy, S.K. Drake, B.J. Wood, M.R. Dreher, Synthesis and in vitro evaluation of cyclic NGR peptide targeted thermally sensitive liposome, J. Control. Release 143 (2010) 265–273.
[106] J. Chen, H. Wu, D. Han, C. Xie, Using anti-VEGF McAb and magnetic nanoparticles as double-targeting vector for the radioimmunotherapy of liver cancer, Cancer Lett. 231 (2006) 169–175.
[107] R.M. Sawant, J.P. Hurley, S. Salmaso, A. Kale, E. Tolcheva, T.S. Levchenko, V.P. Torchilin, "SMART" drug delivery systems: double-targeted pH-responsive pharmaceutical nanocarriers, Bioconjug. Chem. 17 (2006) 943–949.
[108] V. Torchilin, Tumor delivery of macromolecular drugs based on the EPR effect Adv. Drug Deliv. Rev. (in press), doi:10.1016/j.addr.2010.03.011.
[109] J. Fang, H. Nakamura, H. Maeda, The EPR effect: unique feature of blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect Adv. Drug Deliv. Rev. (in press), doi:10.1016/j.addr.2010.04.009.
[110] M. Wang, M. Thanou, Targeting nanoparticles to cancer, Pharm. Res. 62 (2010) 90–99.
[111] P. Ruenraroengsak, J.M. Cook, A.T. Florence, Nanosystem drug targeting: facing up to complex realities, J. Control. Rel. 141 (2010) 265–276.