Advanced Drug Delivery Reviews 97 (2016) 111–143

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

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

Advancement in integrin facilitated drug delivery☆

Daniela Arosio [a,1], Cesare Casagrande [b]

[a] Istituto di Scienze e Tecnologie Molecolari (ISTM), CNR, Via C. Golgi 19, I-20133 Milan, Italy
[b] Università degli Studi di Milano, Dipartimento di Chimica, Via C. Golgi 19, I-20133 Milan, Italy

ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form 27 November 2015
Accepted 3 December 2015
Available online 10 December 2015

Keywords:
Cancer
Integrins
ECM proteins
Peptidomimetics
RGD
Drug delivery
Nanoparticles

ABSTRACT

The research of integrin-targeted anticancer agents has recorded important advancements in ingenious design of delivery systems, based either on the prodrug approach, or on nanoparticle carriers, but for now, none of these has reached a clinical stage of development. Past work in this area has been extensively reviewed by us and others. Thus, the purpose and scope of the present review is to survey the advancement reported in the last 3 years, with focus on innovative delivery systems that appear to afford openings for future developments. These systems exploit the labelling with conventional and novel integrin ligands for targeting the interface of cancer cells and of endothelial cells involved in cancer angiogenesis, with the proteins of the extracellular matrix, in the circulation, in tissues, and in tumour stroma, as the site of progression and metastatic evolution of the disease. Furthermore, these systems implement the expertise in the development of nanomedicines to the purpose of achieving preferential biodistribution and uptake in cancer tissues, internalisation in cancer cells, and release of the transported drugs at intracellular sites. The assessment of the value of controlling these factors, and their combination, for future developments requires support of biological testing in appropriate mechanistic models, but also imperatively demand confirmation in therapeutically relevant *in vivo* models for biodistribution, efficacy, and lack of off-target effects. Thus, among many studies, we have tried to point out the results supported by relevant *in vivo* studies, and we have emphasised in specific sections those addressing the medical needs of drug delivery to brain tumours, as well as the delivery of oligonucleotides modulating gene-dependent pathological mechanism. The latter could constitute the basis of a promising third branch in the therapeutic armamentarium against cancer, in addition to antibody-based agents and to cytotoxic agents.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Extracellular Matrix (ECM) and ECM-like materials: Therapeutic Tools and Targets in Cancer Treatment".

E-mail addresses: daniela.arosio@istm.cnr.it (D. Arosio), cesare.casagrande@tin.it (C. Casagrande).
[1] Tel.: +39 0250314064; fax: +39 0250314072.

http://dx.doi.org/10.1016/j.addr.2015.12.001
0169-409X/

1. Introduction

Integrins are a family of adhesion molecules constituted of 24 heterodimeric transmembrane proteins, which are expressed in a wide range of cells. They play a key role in adhesion to proteins of the extracellular matrix and/or to other cells, essential in physiological development, maintenance, and repair of tissues, as well as in pathological processes in various diseases, specifically in cancer. In fact, the interface of integrin proteins with their counter-receptor proteins, either constituents of the extracellular matrix or expressed in the membrane of other cells, is the site of attachment and localisation, but even more the starting point of active intracellular signalling, leading to cell proliferation, differentiation, and motility towards metastatic sites, in the case of cancer [1–3]. Therapeutic applications of natural and synthetic molecules interfering in integrin-driven pathological mechanisms, and possibly hindering disease progression, have prompted intensive research in laboratory and clinical investigations, leading to proofs of value of the approach and the approval of some drugs for human use [4–6]. It is however noticeable that no anticancer drug is licensed up to now, and of the four approved drug, an antibody, natalizumab, acting on integrin $\alpha_4\beta_1$, is employed in multiple sclerosis, and the group of three antithrombotic agents, acting on integrin $\alpha_{IIb}\beta_3$, consists of an antibody, abciximab, a peptidomimetic, eptafibatide, and a non-peptide, tirofiban. Furthermore, none of the investigational drugs undergoing phase III clinical trial is aimed at anti-cancer application [6], following the withdrawal of the $\alpha_v\beta_3$ antagonist cilengitide [7].

Perhaps this not so encouraging picture, along with some questions on antagonistic vs. agonistic effects [8,9] of molecules endowed with affinity for the integrins ("integrin ligands") has re-addressed the research on integrins towards a deeper understanding of their mechanisms and physiopathological role in cancer, in the hope of novel therapeutic openings. This trend involves their interaction with the proteins of the extracellular matrix and particularly with proteins and cells of tumour stroma [10,11].

On the other hand, the mainstream of therapeutically oriented research on integrin ligands reaffirms the purpose of developing delivery systems for active drugs, diagnostic tools, or both ("theranostics"). In the present review we intend to survey the recent advancement in this direction, focussing on the integrins involved in the interactions of their expressing cells with proteins of the extracellular matrix (ECM). Table 1 lists the integrins that have been targeted for drug delivery, along with their cellular expression on one side, and their adhesion counterparts in the matrix on the other. The latter are represented in some cases by a single protein, but frequently by promiscuous affinities. The table also lists the amino acid sequences ("motifs") of the ECM proteins that specifically drive recognition and binding of the integrins, followed by cell attachment and metastatic proliferation, in the case of cancer. The recognition of the widely expressed sequence RGD (Arg-Gly-Asp), favouring strong attachment by its bipolar functions, is subtly regulated by the sterical conformation, leading to selective interaction with sites of different proteins. Integrins having their adhesive counterparts in other cells, rather than in ECM, are not considered here, except $\alpha_4\beta_1$, having VCAM-1 as its principal counterpart. Also not considered are integrins not yet targeted in delivery system, such as beta2 integrins, and some beta1 integrins.

Main involved ECM proteins [1,2] are: fibronectin (FN), vitronectin (VN), collagens (CO), laminins (LM), tenascin C (TNC), osteopontin (OP), periostin (PO), thrombospondin (TSP), fibrinogen (FG), fibrin(FB), and von Willebrand factor (vWF). Some proteins are soluble and

Table 1
Targeted integrins and their ECM interactions [1–3,6,12,13].

Integrin	Cellular expression	ECM ligands	Binding motifs
$\alpha_v\beta_3$	Endothelial cells; some tumour cells (glioblastomas, melanomas, ovarian, breast and prostate cancers); monocytes; osteoclasts; fibroblast; platelets	FN; VN; LM; FG; Fibrin; TSP; TN-C, vWF; CO; OP; PO; MMP-2; Del-1; BSP; FGF-2; Thrombin, CCN1	RGD
$\alpha_v\beta_5$	Endothelial cells; some tumour cells; fibroblasts	VN; Del-1; CCN1; PO	RGD
$\alpha_v\beta_6$	Epithelial cells; some tumour cells (endometrial, basal cell carcinoma, colon, gastric, cervical, squamous cell carcinoma, oral, pancreas, breast, ovary)	FN; VN; TN-C and the latency associated peptide (LAP) of TGF-β	RGD
$\alpha_2\beta_1$	Endothelial cells; platelets; leukocytes; some cancer cells (prostate, bone metastases)	CO; LM; E-cadherin; MMP 1	DGEA; GFOGER
$\alpha_4\beta_1$	Leukocytes	VCAM-1[a]; FN; OP	LDV
$\alpha_5\beta_1$	Endothelial cells; platelets; lymphocytes	FN; FB	RGD; PHSRN
$\alpha_{IIb}\beta_3$	Platelets	FN; VN; FG; vWF; CD40L; prothrombin; TSP	RGD

First column: main integrin heterodimers. Second column: expression in cells. Third column: ECM proteins interacting with the integrins. Fourth column: binding peptide sequences (when known) recognised by integrins in ECM proteins.
Abbreviations: BSP = Bone sialo protein; CCN1 = Cysteine rich protein 61; CO = Collagen; Del-1 = Developmental locus-1; ECMRVI = Extracellular matrix receptor VI; FB = Fibrin; FG = Fibrinogen; FN = Fibronectin; FNR = Fibronectin receptor; LM = Laminin; OP = Osteopontin; PO = Periostin; TN-C = Tenascin C; TSP = Thrombospondin; VN = Vitronectin; VNR = Vitronectin receptor; and vWF = von Willebrand factor.
[a] VCAM-1 = Vascular cellular adhesion molecule 1, is expressed in endothelial cells.

circulate in plasma, whereas others are less soluble or insoluble and are constitutive of the basement membrane, e.g. laminins, and of connective tissues, e.g. type I collagen and type III collagen. Fibronectin is both a circulating and an insoluble component of ECM. In tumour stroma, collagen I and III, tenascin C, fibronectin isoforms, and periostin have important roles along with CAF (cancer-associated fibroblasts), the main cellular component of the stroma, expressing integrin $\alpha_5\beta_1$ and $\alpha_{11}\beta_1$ [10,11].

The status of research on the targeting of integrins for drug delivery in cancer and in vascular diseases, either by carriers with direct conjugation of an integrin ligand and a drug ("prodrugs"), or by a number of different types of nano- and micro-particles, loaded with appropriate drugs and exposing an integrin ligand on their surface, has extensively been reviewed [7,14–16].

We had to outline the conclusion [14] that clinical developments were not reached with any of these delivery systems, notwithstanding 15 years of research efforts elapsed from the seminal paper of Arap, Pasqualini and Ruoslahti [17]. It was pointed out that many biological studies were limited to *in vitro* experiments, and were not coping, by *in vivo* animal studies, with the problems of biodistribution, pharmacokinetics, tolerability, effectiveness, and advantage over non-targeted delivery systems. We intend now to analyse critically the progress registered in the field in the last 3 years, pointing out the studies reaching a sound proof-of-concept *in vivo*, as needed for progressing to advanced investigations and potential applications.

The debate of the value of targeting adhesion molecules in cancer for effective intracellular delivery to the intracellular targets of the transported drug remains open. Indeed, the actual value of the targeting of nanoparticles has been discussed [18]. The mechanisms of integrin-mediated uptake of small particles have been reviewed [19]. The enhanced intracellular transport of integrin-targeted, with respect to non-targeted nanoparticles is also a matter of discussion [14,20–23].

Again, in recent studies, [24,25] integrin ligands of the RGD type have been reported to facilitate the transport of drugs into cells by endocytosis through multiple pathways, including caveolae- and chlatrin-mediated endocytosis, as well as macropinocytosis, in malignant U87MG glioma cells, in cellular spheroids, and in subcutaneous or intracranial mouse xenotransplants.

Still, a favourable effect of integrin targeting on intracellular delivery is not granted [26–28]. An important question, with different answers offered in recent papers, is whether the targeting moieties shall be presented on the outer layer of a PEGylated delivery system. As an alternative, the system can be covered by biochemically removable PEG sequences, thus providing the EPR effect [29,30], but then uncovering the targeting moieties, and/or other moieties favouring internalisation and intracellular effects in cancer cell. To answer the question, finding the way to reach the intracellular target, is a stimulating challenge of current research. It has to be remarked that the ingenuity applied in facing the challenge frequently demands sophisticated manipulations in the construction of the delivery systems. This can increase difficulties of practical feasibility, which is necessary for development and clinical application, not the least from the point of view of cost–benefit ratio [31].

In the first sections we analyse the recent progress in the discovery and the synthesis of new ligands of $\alpha_v\beta_3$ integrin, as well as of other integrins less extensively investigated, and also the progress in the design of new prodrugs, as well as new particulate systems and use of new materials, rather than classical nanoparticles. Next, we focus on the innovative approaches in improving, in addition to targeting, internalisation in tumour cells. A section is dedicated to the exploitation of mechanisms of activation for the release of the active drug, either by taking advantage of biochemical mechanism peculiar of cancer cells, and/or by the use of external physical stimuli suitable for imaging the delivery, or for inducing drug release, or for physical effect of inhibition of tumour growth. Some of these delivery systems, involving multiple approaches pertain to the field of "theranostics", are discussed in the *ad hoc* section, although many appear useful as investigational tools, and not for actual development in therapy. Finally, in the last two sections, we focus on two subjects of high therapeutic relevance. The first is the treatment of brain tumour and relevant methods of transport across the blood brain barrier, the latter the delivery of oligonucleotides for modulation of pathologically relevant genes, which could in future represent an alternative to cytotoxic agents.

2. New ligands for integrin receptor targeting

2.1. Integrin $\alpha v\beta 3$

Most efforts in search of integrin receptor ligands have been addressed to α_v integrins, mainly $\alpha_v\beta_3$, (sometimes to the related $\alpha_v\beta_5$), yielding a variety of high affinity peptides, peptidomimetic, and non-peptide ligands, all mimicking the RGD moiety of various ECM proteins [32–35]. The role of this integrin in angiogenesis and tumour cell proliferation is well-documented [1–3]. While it is barely detectable in quiescent vessels and normal endothelium, it is highly expressed in new blood vessels of tumours, and in cells of several solid tumours, such as glioblastomas, melanomas, ovarian, breast and prostate cancers. Levels of expression correlate with tumour growth, invasiveness and metastatic capacity. Among the many ligands, a few structures provide functional groups suitable for chemical conjugation. Thus, most of the

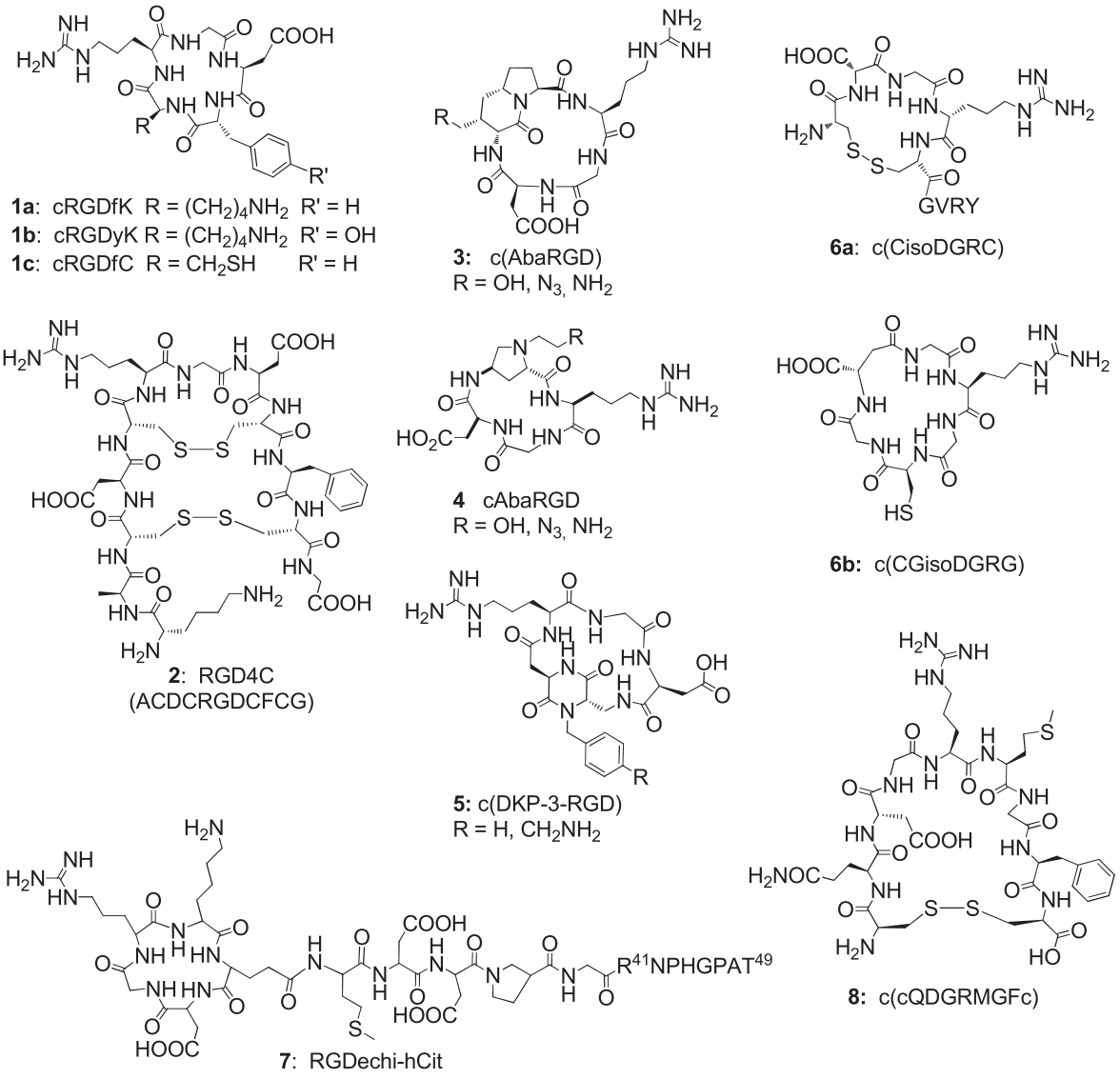

Fig. 1. Peptidic and peptidomimetic ligands used for targeting integrin $\alpha_v\beta_3$.

studies aiming at prodrugs or at delivery systems of anticancer drugs exploited three "cilengitide-like" cyclopentapeptides, cRGDfK (1a, Fig. 1) and cRGDyK, (1b, Fig. 1) where the amino group of lysine can be conjugated by amidation, and cRGDfC (1c, Fig. 1), where the thiol group of cysteine residue is suitable for Michael addition of maleimide-functionalised linkers. Another ligand, an undecapeptide from a phage display library, RGD4C (2, Fig. 1) [17], conformationally constrained by two disulphide bridges, is used in delivery systems by conjugation at its N- or C-terminals [17,22,36–39]. New approaches with RGD4C are represented by its expression in recombinant proteins, suitable for incorporation in integrin targeted systems, or sometimes directly as targeted drugs [40–42] as in the case of the RGD4C-TNF fusion protein developed by Wang *et al.* by recombinant DNA technology for the delivery of tumour necrosis factor (TNF) [40]. RGD4C-TNF was chemically conjugated with DOTA and labelled with the positron emitter ^{64}Cu isotope. The system allowed microPET correlation of its uptake in various tumour models *in vivo* with the level of integrin expression, and proved in MDA-MB-435 xenografts enhanced inhibition of tumour growth with respect to non-targeted TNF (72% inhibition vs. 24%). Xie and co-workers expressed RGD4C in genetically modified ferritin nanocages, that were loaded with the photosensitiser zinc hexadecafluorophthalocyanine (ZnF16Pc) [41] or with DOX [41]. Both constructs were tested in U87MG subcutaneous tumour models, the former showing high accumulation and the latter inhibition of tumour growth (see Section 4.5.1).

A different class of ligands, using rigid peptidomimetic scaffolds (bicycloalkanes, aminoprolines, diketopiperazines, etc.) for blocking the RGD recognition sequence in active and selective conformations for integrin $\alpha_v\beta_3$, is now enriched with functions for conjugation. Our group has reported ligands based on an azabicycloalkane (Aba) scaffold (3, Fig. 1) [43] conjugated with imaging probes such as fluorescein [44] and Cy5.5 [45] or with chelating agents for radionuclides or lanthanides [46,47], with encouraging results in tumour detection *in vivo*. The prodrug obtained by conjugation with PTX showed, in an IGROW1-Pt xenograft model, enhanced efficacy on tumour growth inhibition, with respect to free PTX administered at twice the dose [48]. Similar behaviour was observed with a prodrug displaying multiple ligands [49]. Labelling with azabicycloalkane (Aba)- and aminoproline (Amp)-based ligands (3 and 4, Fig. 1) was used for delivering DOX-loaded liposomes (LNPs). Flow cytometry and fluorescent microscopy, showed that labelled LNPs delivered DOX in the nuclei of both quiescent and proliferating MCF7 cells of human breast adenocarcinoma and induced cell death, with 2- to 6-fold increased potency with respect to free DOX or non-labelled DOX-liposomes [50].

Along the same lines, Gennari and co-workers conjugated diketopiperazine (DKP)-RGD ligands with PTX. The prodrug 5 (Fig. 1),

endowed with adequate stability in plasma, was tested *in vivo* in an IGROV-1/Pt xenograft model in mice, showing decreased tumour growth with respect to free PTX [51]. In a subsequent study a protease-sensitive dipeptide linker, instead of an ester linkage, was introduced in order to modulate enzymatic PTX release (Section 3.1) [52].

The ligand *Cisо*DGRC (Cys-isoAsp-Gly-Arg-Cys) (6a, Fig. 1) is a conformationally restricted analogue of *iso*DGR. The latter is formed by spontaneous deamidation–isomerisation of NGR (Asn-Gly-Arg), a peptide exploited for targeting aminopeptidase N-expressing ($CD13^+$) tumour cells. At difference from NGR, *Cisо*DGRC has remarkable affinity for $\alpha_v\beta_3$ [53]. Synthetic CDAK peptide (*Cisо*DGRCLLIIKLAKLAKKLAKLAK), combining features of *Cisо*RGDC and antimicrobial peptides, demonstrated antitumour effect in $CD13^-/\alpha_v\beta_3^+$ breast cancer cells both *in vitro* and *in vivo* [54]. Another ligand of this type, c(CG*isо*DGRG) (6b, Fig. 1)[55], selected in a set of synthetic analogues for its best $\alpha_v\beta_3$-selectivity, was conjugated to human serum albumin (HSA) (Section 4.5.4), and investigated on cells and, *ex vivo*, on tumour specimens for targeting gold nanoparticles carrying both the albumin-conjugate and TNF on their surface. These gold particles showed *in vivo*, in WEHI fibrosarcoma-bearing mice, a significant delay of tumour growth. Authors suggest that c(CG*isо*DGRG)-HSA may open the way to a novel class of albumin-based targeted delivery systems.

The $\alpha_v\beta_3$ ligand, RGDechi-hCit [56,57] (7, Fig. 1) was designed by superimposing the NMR structure of echistatin on the structure of the $\alpha_v\beta_3$/c(RGDf[NMe]V) complex. Two selected sequences of echistatin, Met28-Asp30 and Arg 41-Thr 49, were connected by a Pro-Gly linker and coupled via Met with the gamma-carboxylic end of c(KRGDe) [58]. The construct was able to penetrate into human malignant melanoma WM266 cells by clathrin- and caveolin-mediated endocytosis, and showed intrinsic proapoptotic effects, thus prospecting application in delivery systems.

By a combinatorial one-bead one-compound technique, Zhang *et al.* selected c(cQDGRMGFc) peptide (8, Fig. 1) containing the DGR recognition motif [59] and showed its ability of binding to a set of bladder cancer cell lines, as well as primary cells from human tumours, but not to normal urothelial cells or cell mixtures from normal bladders. Sequential alanine shift of each residue showed that D, R, or F replacement suppressed binding to 5637 cells, indicating integrin $\alpha_v\beta_3$ as the target. The peptide was employed in delivery micelles loaded with PTX or daunorubicin to bladder cancer (see Section 7.3) [60,61].

The search for novel ligands is actively progressing for other α_v type integrins, and other integrins recognising the RGD sequence in ECM proteins, such as the fibrinogen receptor $\alpha_{IIb}\beta_3$, and importantly for $\alpha_5\beta_1$, the fibronectin receptor. Ongoing progress is also targeting ECM molecular expressions not related to RGD [4].

2.2. Integrin $\alpha_v\beta_6$

Integrin $\alpha_v\beta_6$, exclusively expressed in epithelial cells, is a receptor for the latency associated peptide (LAP) of TGF-β and for the (ECM) proteins fibronectin, vitronectin, and tenascin. Although barely detectable in normal epithelia, it is upregulated during wound healing and in different cancers (colon, ovarian, endometrial, and gastric) and often associates with a poor prognosis. It was shown to promote cell invasion and migration in metastasis, and to inhibit apoptosis. This integrin is attractive as tumour biomarker and potential therapeutic target, and for its role in expression of matrix metalloproteases (MMPs) and activation of TGF-β1 [13].

A 20-mer peptide (named H2009.1; 9 Fig. 2), with high-affinity and specificity for the integrin $\alpha_v\beta_6$, was identified by Brown and co-workers in phage-displayed library [62]. Tetramerisation of H2009.1, or of the corresponding N-terminal decamer (10, Fig. 2) on a trilysine–dendrimer core improved the affinity with respect to the monomers [62]. The constructs were conjugated with anticancer agents, e.g. hydrazone-bond linked DOX (see Section 3.2), [63], or PTX [64]. The latter showed comparable effects, although somewhat delayed, with free PTX in a H2009 xenograft model in mice [64].

The H2009.1 peptide was employed in other delivery systems. It was displayed on multifunctional micelles encapsulating superparamagnetic iron oxide NPs (SPIONs) and DOX. *In vitro* imaging of $\alpha_v\beta_6$-expressing H2009 cells by MRI and by radioactivity (using ^{3}H-labelled micelles), showed significantly enhanced targeting and uptake in comparison with control micelles carrying a scrambled peptide [65]. DOX cytotoxic effects were also increased.

The contributions of label multiplicity and density in DOX-liposomes labelled with H2009.1 (11 and 12, Fig. 2) were tested *in vitro* on H2009 cells, showing 5-10 fold higher drug delivery with the tetrameric peptide than with the monomer, even when the same total number of subunits was displayed. Targeting and cytotoxicity also increased proportionally with the amount of tetramer [66]. Unfortunately, all H2009.1-labelled liposomes exhibited the same efficacy *in vivo* and did not differ from non-labelled controls, indicating that, in the instance, *in vivo* accumulation in tumours may be mainly controlled by the EPR effect [28].

2.3. Integrin $\alpha_5\beta_1$

Similarly to $\alpha_v\beta_3$, $\alpha_5\beta_1$ integrin plays a fundamental role in tumour angiogenesis, being poorly expressed on normal quiescent vessels, but up-regulated in tumour blood vessels [1]. $\alpha_5\beta_1$ selectively binds fibronectin primarily through an RGD recognition motif, but also binds to a "synergy binding sequence" PHSRN of fibronectin, which can be targeted with specific ligands. One of these, ATN-161 (Ac-PHSCN-NH_2), was investigated in phase II trials in cancer [6].

Kessler and co-workers investigated sets of non-peptidic integrin ligands for selective binding and inhibition of either $\alpha_v\beta_3$ or $\alpha_5\beta_1$ integrins [67–69]. Compounds 13a and 14a (Fig. 3) respectively showed selective efficacy in mediating cell adhesion of $\alpha_v\beta_3$ or $\alpha_5\beta_1$-expressing fibroblasts [67]. By conjugation with imaging moieties (^{68}Ga-13b and ^{68}Ga-14b, Fig. 3) *in vivo* experiments respectively showed uptake in mice bearing RKO human colon carcinoma, with high $\alpha_5\beta_1$ expression, or in mice bearing M21 human melanoma, highly expressing $\alpha_v\beta_3$. In mice bearing syngeneic subcutaneous WEHI-164 fibrosarcomas, unconjugated compounds 13a and 14a showed comparable delays in tumour growth, indicating similar antitumour efficacy either by inhibition of $\alpha_v\beta_3$ or $\alpha_5\beta_1$ [68].

Dai *et al.* [70] labelled DOX-loaded liposomes with peptide PHSCNK for targeting $\alpha_5\beta_1$ integrin on tumour vasculature. Experiments of uptake and cytotoxicity on HUVECs and MDA-MB-231 breast cancer cells showed enhanced effects, ascribed to integrin mediated endocytosis, with respect to non-labelled liposomes.

In a search of potent and selective ligands for integrin $\alpha_5\beta_1$, Kokkoli *et al.* designed a peptide, denominated PR_b (Fig. 4A), connecting the RGDSP sequence with the synergy binding sequence PHSRN through an SG_5 spacer [71]. This peptide, was employed in labelling stealth liposomes (LNPs) encapsulating 5-fluorouracil, using variable surface densities of the ligand and of PEG. Best targeting, in mouse CT26.WT colon cancer cells, was achieved with high peptide density, whereas it decreased with high PEG density. The LNPs showed equal cytotoxicity with free 5-FU, and were significantly more effective than GRGDSP-labelled or non-labelled LPNs [72]. PR_b labelled LNPs and polymer NPs were also equipped for delivery to $\alpha_5\beta_1$-rich tumour cells of various cytotoxic drugs, such as DOX [73] and cisplatin [74] or nucleic acids such as siRNA (siOrai3) [75], DNA (luciferase) [76] and plasmid DNA (pNF-κB-Luc) [77] to different $\alpha_5\beta_1$-rich tumour cell lines. The results indicated either increased cytotoxicity of loaded drugs [73,74] or improved transfection efficiency in case of nucleic acids [75–77], suggesting the interest of *in vivo* investigation in both directions.

Wang *et al.* employed the PR_b developed by Kokkoli's group in a platform combining Pc 4 (a second-generation photodynamic therapy drug) with iron oxide nanoparticles (IONPs) [78]. Water-soluble

9 H2009.1 20-mer: NH_2-RGDLATLRQLAQEDGVVGVR-$CONH_2$
10 H2009.1 10-mer: RGDLATLRQL-$CONH_2$

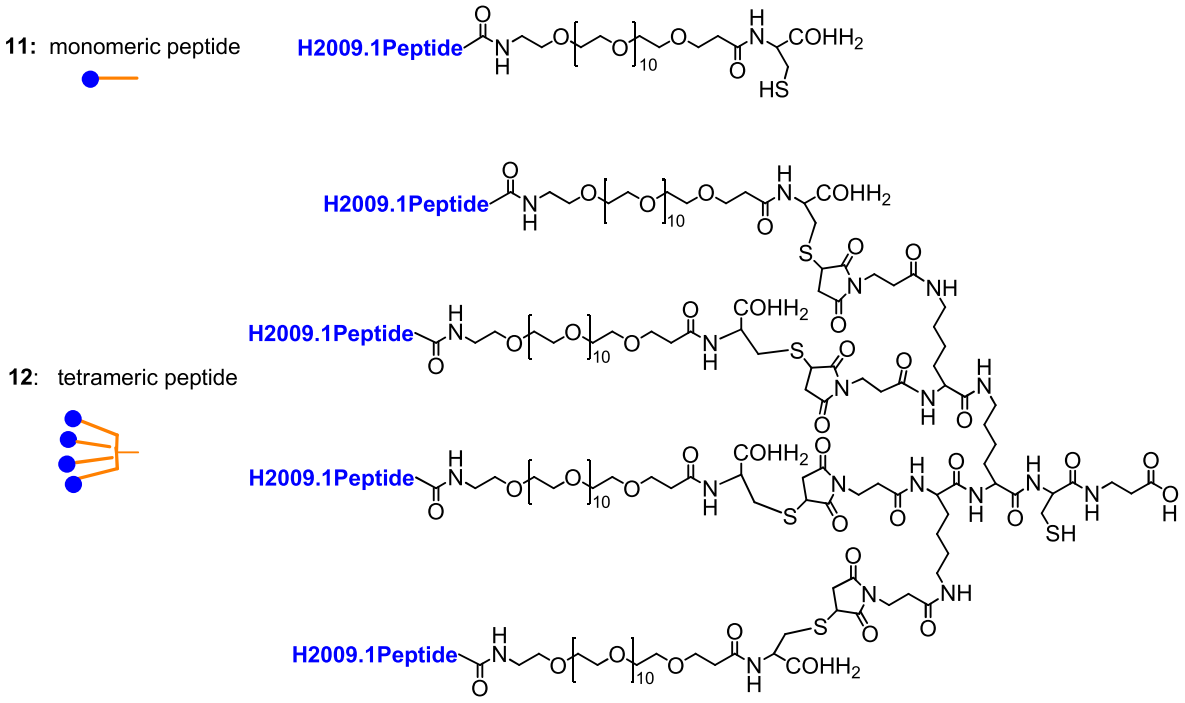

Fig. 2. 20-amino-acid peptide 9 and N-terminal decameric ligand 10 ligands of integrin $\alpha_v\beta_6$. [62], and monomeric and tetrameric constructs 11 and 12 applied for liposome functionalisation [28,66].

IONPs were obtained by encapsulation in a polymer carrying carboxylic groups that also allowed the conjugation of the targeting peptide and encapsulation of Pc 4. MRI imaging was performed *in vitro*. *In vivo*, in mice xenografts of human HNSCC M4E tumour, both non-targeted and labelled Pc 4-loaded NPs accumulated in tumours and reduced tumour size more effectively than free Pc 4. Still, at 8-fold lower doses,

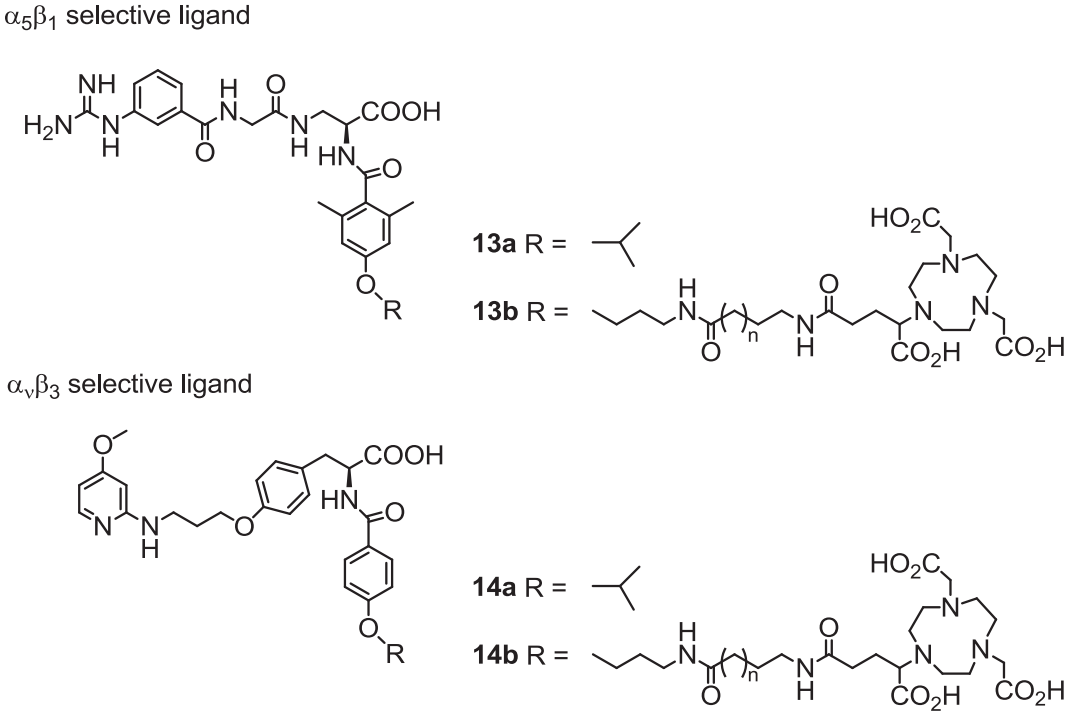

Fig. 3. Small, non-peptidic integrin antagonists with selective binding and inhibition of either $\alpha_v\beta_3$ or $\alpha_5\beta_1$ integrins [67–69].

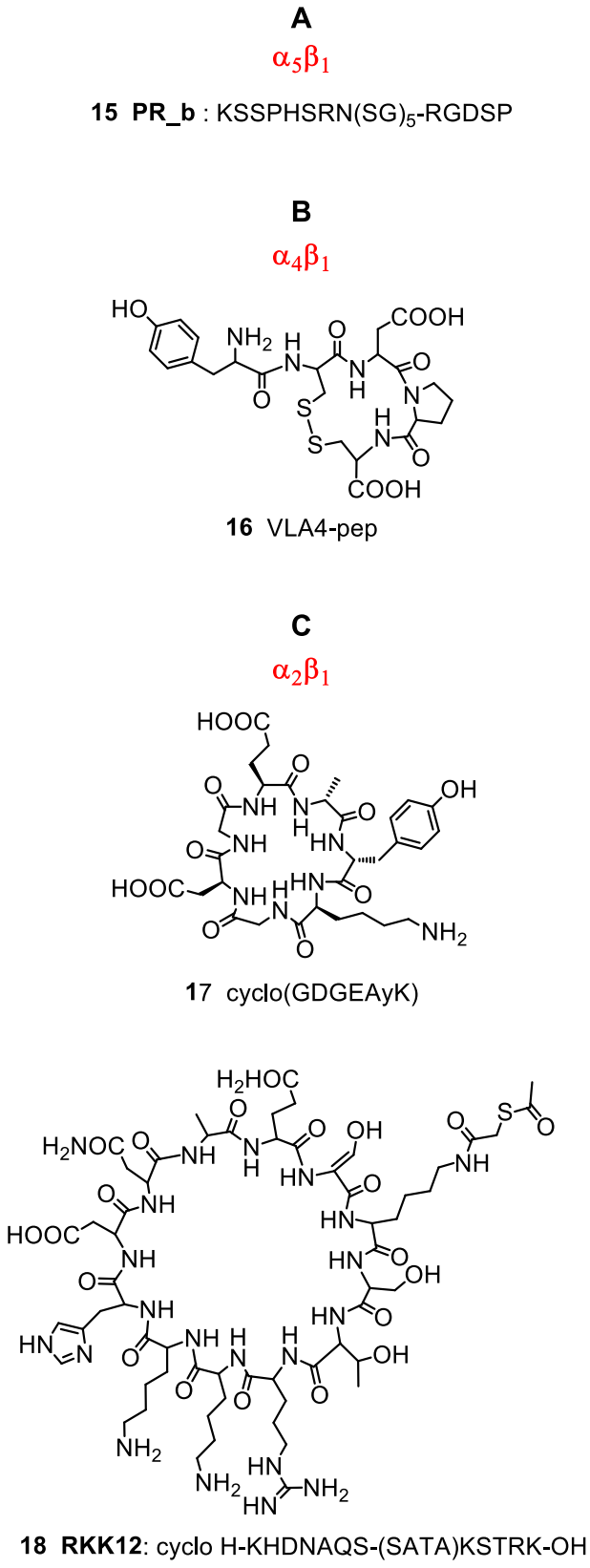

Fig. 4. Ligands targeting other integrins. A) 15: PR_b (KSSPHSRN(SG)5-RGDSP) peptides targeting integrin $\alpha_5\beta_1$ [71]. B) 16: VLA4-pep cyclo(Tyr-Cys-Asp-Pro-Cys) targeting integrin $\alpha_4\beta_1$ [79–81]. C) 17: cyclo(GDGEAyK) [86] and 18: RKK12 (cyclo H-KHDNAQS-(SATA)KSTRK-OH) [88,89] peptides targeting integrin $\alpha_2\beta_1$.

targeted NPs showed significantly greater inhibition of tumour growth than non-targeted ones.

2.4. Integrin $\alpha_4\beta_1$

Integrin $\alpha_4\beta_1$, also known as very late antigen 4 (VLA-4), is highly expressed in leukocytes and specifically in lymphocytes and mediates adhesion and extravasation by binding the cellular counter-receptor VCAM-1 on inflamed endothelial cells. However, the integrin is also expressed on the neovessels of some tumours and on the cell surface of cancers of haematopoietic origin, such as lymphomas, leukaemias, and multiple myeloma, and plays a role in cellular attachment and proliferation by binding to fibronectin [1].

Bilgicer and co-workers identified the small peptide Tyr-Cys-Asp-Pro-Cys (namely VLA4-pep, Fig. 2B) as a high affinity ligand for $\alpha_4\beta_1$-expressing multiple myeloma cells [79], and exploited it in labelling micelles carrying hydrazone-linked DOX (see Section 3.2). These NPs efficiently internalised and induced cytotoxicity in multiple myeloma cells. Moreover, in cells cultured on fibronectin-coated plates, they were able to overcome the drug resistance induced by the adhesion, as shown by increased toxicity with respect to free DOX. *In vivo*, in a multiple myeloma xenograft, targeted DOX-loaded micelles preferentially accumulated in tumours with respect to free DOX and non-targeted particles, and inhibited tumour growth with less systemic toxicity. The same group also reported the optimisation of ligand density, hydrophilicity, and PEG linker length in micelles and liposomes [80,81].

2.5. Integrin $\alpha_2\beta_1$

Integrin $\alpha_2\beta_1$, also known as VLA-2, GPIa-IIa, and CD49b, is a receptor for ECM collagens and laminins. It is expressed on T and B lymphocytes, platelets, fibroblasts, endothelial cells and on various cancer cells, including melanoma. Though integrin $\alpha_2\beta_1$ knockout mice have no relevant defects in development or viability, indicating no fundamental role in embryo angiogenesis, it has relevant role in tumour angiogenesis [1].

The minimal active recognition sequence DGEA, corresponding to residues 435-438 of the type I collagen sequence [82], was employed for targeting integrin $\alpha_2\beta_1$ in prostate cancer, where its expression often correlates with increased progression and invasiveness. Favourable *in vivo* imaging of conjugated DGEA -NIR and -PET probes [83,84] prompted conjugation with DOX-loaded nanodiamonds. *In vitro* test on $\alpha_2\beta_1$ expressing prostate cancer (PC3) cells, showed moderate increase in cytotoxicity with respect to human mesenchymal stem cells (hMSC) with low integrin expression [85].

Chung *et al.* enclosed the DGEA sequence in the cyclic $\alpha_2\beta_1$ ligand cGDGEAyK (17, Fig. 2C), that they labelled with ^{68}Ga for PET imaging of human glioblastoma U87MG [86]. Targeting specificity was confirmed by competition with the known $\alpha_2\beta_1$ ligand TC-I 15 [87]. Importantly, the ^{68}Ga-peptide was investigated in microbubble disruption experiments *in vivo* in a glioma model (see Section 8) [86].

Cyclopeptide RKK12 (18 Fig. 2C), derived from the Jararhagin protein of snake venoms and containing the Arg-Lys-Lys-His (RKKH) $\alpha_2\beta_1$-specific binding motif, was used by Knudsen *et al.* [88] for conjugation with the distal end of PEGs in PEGylated liposomes. These were loaded with calcipotriol, aiming at the topical treatment of psoriasis [89]. *In vitro* studies on human keratinocyte cells HaCaT showed increased transport of calcipotriol into cells, enhancing the transcription, via D_3-receptor activation, of the gene encoding the antimicrobial peptide cathelicidin.

3. Integrin targeted prodrugs

In general terms, the “prodrug” approach defines the use of reversible molecular modification of active drugs by conjugation with

moieties intended to protect the drugs during biodistribution, to mask their activity in the process, and to enhance the ability to reach the site of action, and then be removed by biochemical mechanism, regenerating on site the active species. General modification, such as those increasing lipophilicity, can assist in trespassing organ and tissue barriers, improving oral absorption, distribution in organs, and protection from first-pass metabolism undesired accumulation in inactive sites. More subtle improvements can be expected by modalities using targeting specific features of the site of action (as in the case of integrins), and/or exploiting the removal of protective moieties with biochemical mechanism specific of target tissues (e.g. pH gradient, reductive or oxidative mechanisms or enzymolysis) [90,91].

Integrin targeting of prodrugs for cancer therapy in the last years provides examples of both modalities [92]. The antagonist properties exerted by many integrin ligands could potentially contribute to the overall effect, by antiangiogenic or other effects. This would result in a bimodal action, corresponding to the so-called "mutual prodrug" approach of conjugating two active drugs in a construct suitable to improve the biodistribution of both. Still, in prodrugs of potent anticancer drugs, the integrin ligand can possibly contribute more by improved site delivery than by intrinsic efficacy.

The potential value of a prodrug can be assessed *in vitro* or, preferably, in *in vivo* models for efficacy and/or biodistribution.

It will be discussed how some investigators proceeded by the inclusion of prodrug in particulate delivery devices, and how conjugation methods employed for prodrug synthesis were also applied for chemical conjugation to nanomaterials. [93–97].

3.1. Paclitaxel prodrugs

Paclitaxel (PTX) prodrugs are mostly synthesised by conjugation at the 2′-hydroxy group. It has been ascertained that irreversible links in this position suppress cytotoxicity of PTX derivatives [98]. PTX was therefore conjugated with integrin ligands through succinate or diglycolic ester functions (19–21, Fig. 4A), with bonds chemically stable but enzymatically cleavable by esterases [48,51,99–101]. The circulating half-life of these esters is often short, and hydrolysis by esterases of plasma or organs can occur before reaching tumour sites [102], thus losing any targeting effect. To overcome esterase cleavage, Gennari and coworkers explored conjugation with esterase insensitive, but protease sensitive dipeptide linkers Val-Ala and Phe-Lys [52], with additional use of a self-eliminating p-aminobenzyl carbonate link for fast release (Fig. 5B). The antiproliferative action of two constructs (23 and 24, Fig. 5B) was compared with that of 22, having stable amide link, *in vitro* in acute lymphoblastic leukaemia cell line CCRF-CEM $\alpha_v\beta_3^+$ and $\alpha_v\beta_3$ devoid CCRF-CEM $\alpha_v\beta_3^-$. Compounds 23 and 24 showed improved antiproliferative activity in the $\alpha_v\beta_3$-endowed line, with 7-fold enhanced effect with respect to free PTX, whereas 22 was inactive in both lines [52].

3.2. Doxorubicin prodrugs

A doxorubicin (DOX) prodrug targeting integrin $\alpha_v\beta_3$ was first reported in 1998 by Arap *et al.* who coupled the 3′-amino position of DOX with the free carboxylic groups of RGD4C by activation with 1-ethyl-3-(3,3-

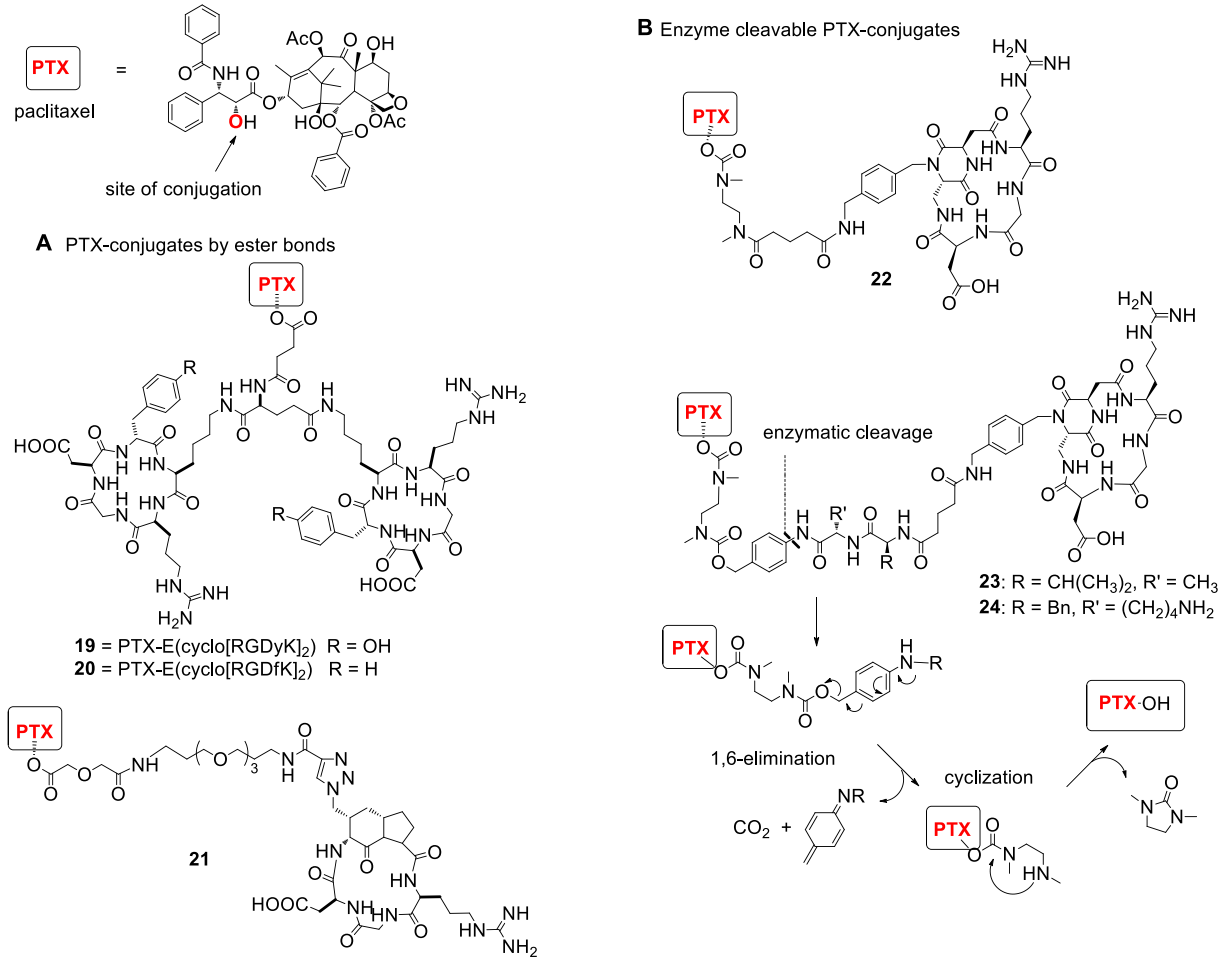

Fig. 5. Integrin targeted paclitaxel (PTX) prodrugs conjugated by: A) ester bonds [48,99–101]; and B) enzyme-cleavable peptide linkers [52].

dimethylaminopropyl) carbodiimide and *N*-hydroxysuccinimide (NHS) [17]. Four treatments in 12 weeks with a sub-optimal dose (corresponding to 30 μg of DOX) in mice bearing human MDA-MB-435 breast carcinoma, resulted in decreased tumour growth and inhibition of metastases in comparison with free DOX. The prodrug appeared to be less toxic for liver and heart than free DOX in *ex vivo* histopathological assays [17]. The experiments were not designed to clarify if the reported effects could be attributed to the peptide-DOX construct or to released DOX.

Proteinase cleavable linkers were used for preferential release in cancer cells with respect to the stable amide link [39,103]. De Groot *et al.* conjugated RGD4C to DOX by the D-Ala-Phe-Lys sequence recognised by the tumour-associated protease plasmin (known to be involved in tumour invasion and metastasis [39]). An aminocaproyl residue was incorporated as a spacer between the RGD4C and the amino-terminal of the tripeptide sequence, whereas a self-eliminating 4-aminobenzyl alcohol spacer was inserted between the plasmin substrate and doxorubicin (25 Fig. 6A). After assessment of cleavage by plasmin, *in vitro* experiments in HT1080 fibrosarcoma cells and in HUVECs, in the presence of plasmin, assessed cytotoxicity, near to the potency of free DOX. Ryppa *et al.* compared integrin targeted DOX conjugates with either an amide link or a proteinase cleavable sequence [103]. They synthesised the stable derivative by coupling thiolated E-[c(RGDfK)$_2$] with maleimido-linked DOX, and the MMP-2/MMP-9 cleavable derivative by inserting the *ad hoc* sequence between integrin ligand and DOX (26 Fig. 6B). The sequence was effectively cleaved, releasing DOX in homogenates of OVCAR-3 human ovarian carcinoma, whereas no release occurred from the amide link. Unfortunately, no significant effects occurred with either conjugates in mice xenografted with OVCAR-3.

In many other examples DOX was conjugated with drugs and polymers to be employed in the preparation of NPs by pH responsive bonds, frequently used because they remain stable near pH 7 but are easily cleaved in the acidic condition of tumour tissues and intracellular compartments. A way of conjugation is through the *cis*-aconityl linker (Fig. 6C), as reported by Wang *et al.*, who used it with the iRGD peptide [104] for improving targeting and penetration of DOX in gliomas (see Section 8) [93]. Zhang *et al.* employed the link for conjugation of DOX on RGD-modified PEGylated polyamidoamine (PAMAM) dendrimers to be use in glioma models (see Section 8) [94].

The hydrazone bond (Fig. 5D) is also pH sensitive and is often employed for conjugation of DOX. Cai, Gong and co-workers employed it for conjugating DOX with PEGylated, cRGDfC functionalised, SPIONs targeting U87MG gliomas (see Section 7.2) [95]. They also provided an amphiphilic block-copolymer, H40-poly(L-glutamate-hydrazone-DOX)-b-poly(ethylene glycol), for conjugation with cRGD and with ^{64}Cu chelating NOTA for U87MG targeting and imaging (see Section 7.2) [96]. Conjugation of DOX via hydrazone link in micelles carrying TAT is also reported (see Section 5.1) [38].

3.3. Camptothecin prodrugs

Dal Pozzo *et al.* employed cRGD ligands with functional groups suitable for conjugation through appropriate linkers with camptothecin (CPT) and its analogues [105]. The linkers where characterised by stable amide (27, Fig. 7A) or pH-sensitive hydrazone bonds (28, 29 Fig. 7A). All the conjugates preserved the affinity for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ isolated receptors, but only the labile ones exhibited cytotoxicity comparable

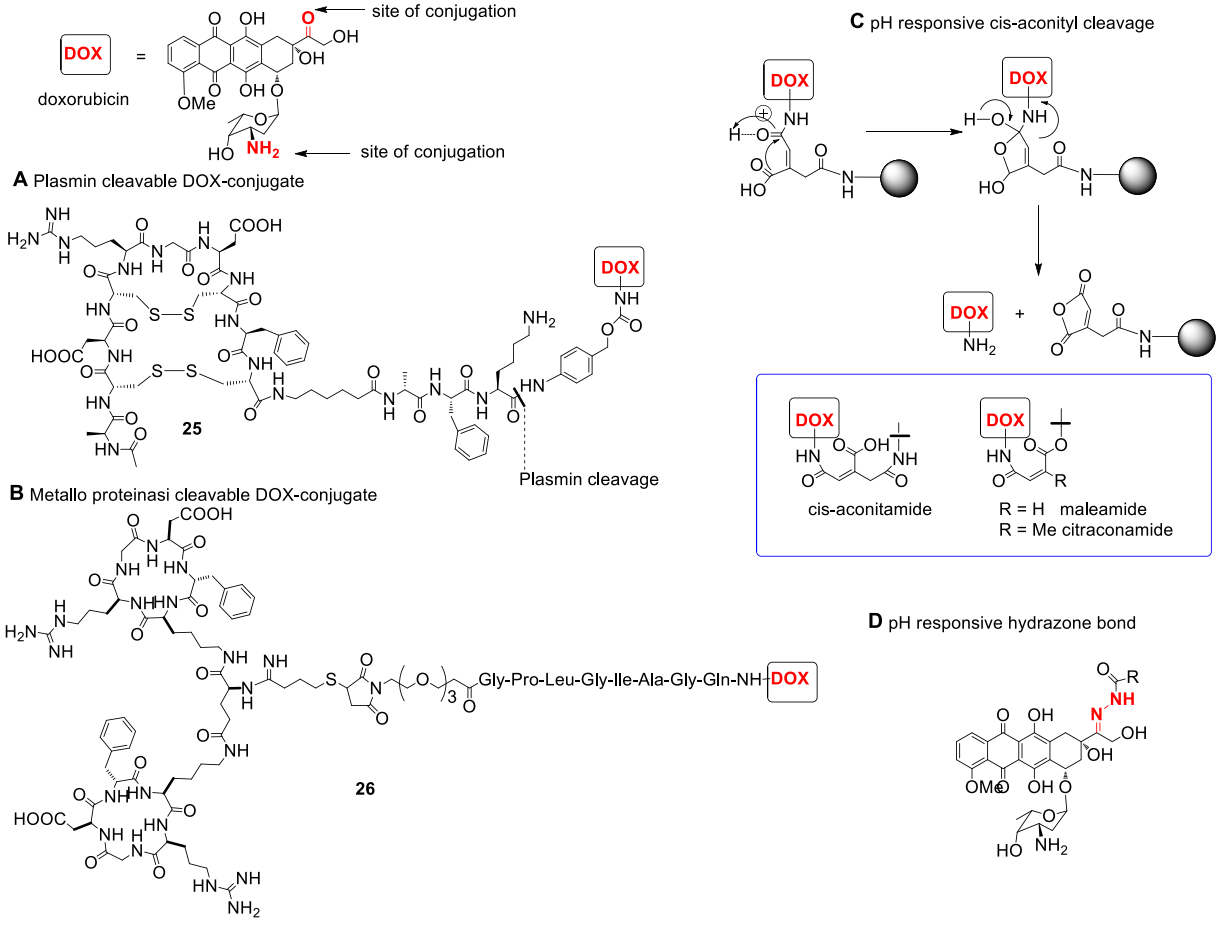

Fig. 6. Integrin targeted DOX prodrugs with conjugation by: A) plasmin cleavable linker [39]; B) MMP cleavable linker [103]; C) pH-responsive *cis* aconityl moiety [93,94]; and D) pH-responsive hydrazone bond [95,96].

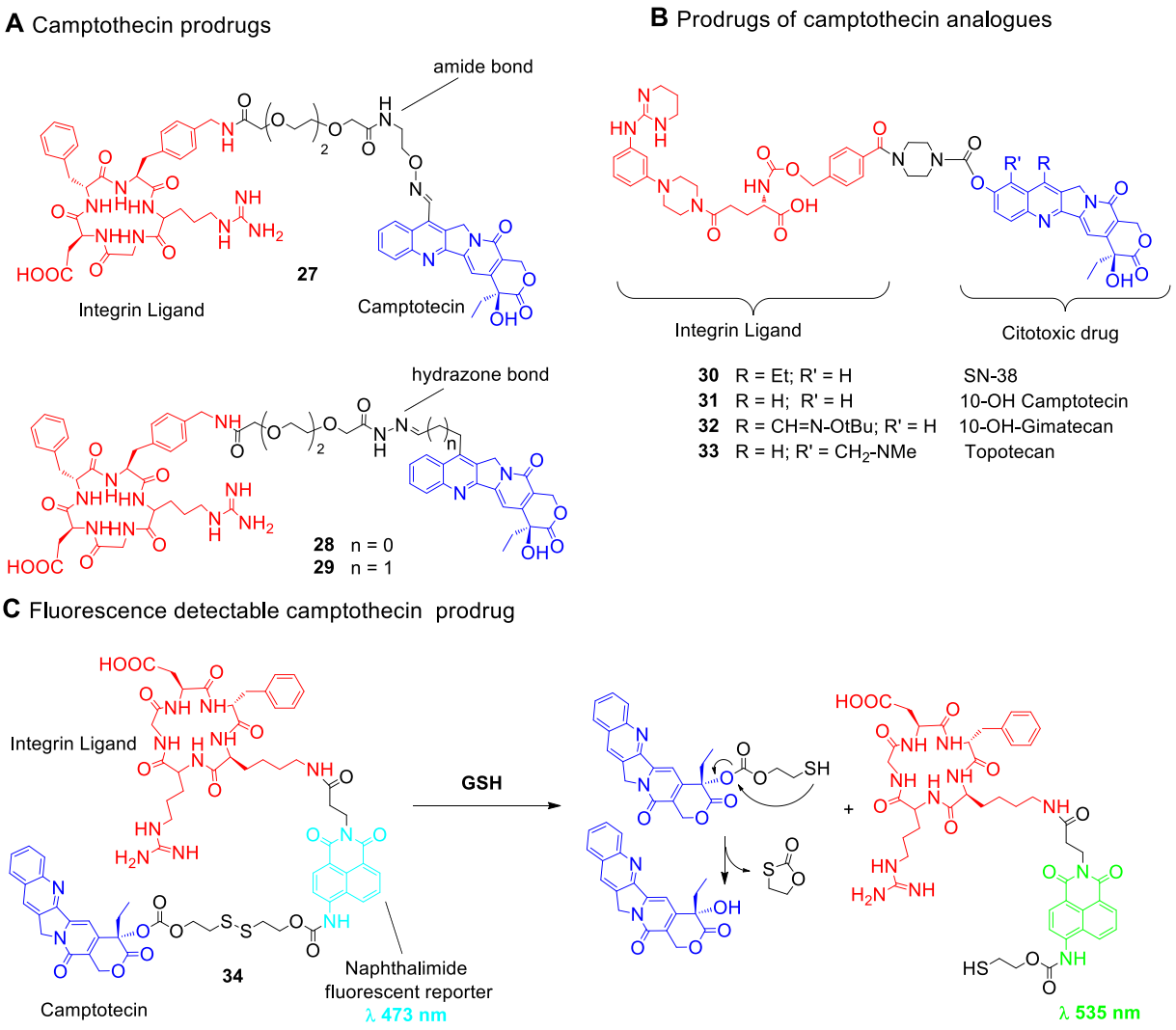

Fig. 7. Campothecin prodrugs: A) CPT-conjugates, either through stable amide or pH-sensitive hydrazone bonds [105]; B) CPT-analogue conjugates with a non-peptide integrin ligand [109]; and C) CPT-conjugates linked by S-S bridge with fluorescence probe [110].

with parent drugs in cells of prostatic (PC3), renal (A498), and ovarian (A2780) carcinomas. *In vivo* evaluation was hampered by low solubility. In a second study, the camptothecin derivative namitecan was linked through lisosomally cleavable dipeptides (Phe-Lys or Val-Cit) [106]. The eight conjugates maintained affinity and cytotoxicity. In particular, two of them, carrying a pair of integrin ligand and endowed with increased affinity towards isolated $\alpha_v\beta_3/\alpha_v\beta_5$ receptors, displayed high cytotoxicity in cells, and good stability in plasma, thus deserving *in vivo* confirmation.

Irinotecan, itself a prodrug belonging to the family of CPTs, has anti-tumour activity due to rapid conversion *in vivo* by human carboxylesterases into the active metabolite SN-38 (10-hydroxy-7-ethyl-CPT) [107]. Giannini and co-workers linked this metabolite and other 10-hydroxy-CPT derivatives with a non-peptide RGD mimetic disclosed by Iwama *et al.* [108] and functionally modified for conjugation [109]. Cytotoxicity of four compounds 30–33 (Fig. 7B) was assessed *in vitro* on human ovarian carcinoma (A2780) cells overexpressing integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$, as well as in a line of prostate cancer cells PC3 with low integrin expression, showing for compound 30 an enhanced antiproliferative activity on A2780 cells. The compound was more stable than irinotecan in plasma, with comparable results in term of plasma life span and tumour growth inhibition in PC3 prostate cancer and A498 renal carcinoma xenografts in mice in comparison with free SN-38.

A fluorescence traceable construct was obtained from CPT by cRGDyK-labelling through a linker consisting of a cleavable S-S bridge and a naphthalimide fluorescence reporter. [110] (Fig. 7C). Preferential uptake in U87 human glioma cells, overexpressing integrin $\alpha_v\beta_3$ with respect to integrin poor C6 rat glioma cells, was assessed by confocal microscopy. Uptake was suppressed by adding the endocytosis inhibitor okadaic acid. Intracellular release, by GSH cleavage of the S-S bridge and collapse of the carbonate ester bond, was detected by red-shift to 535 nm of the fluorescence, which appeared to co-localise with an endoplasmic reticulum-selective dye. Enhanced cytotoxicity with respect to a non-labelled construct was also observed.

3.4. Pt(IV)-prodrugs of platinum(II) chemotherapeutics

A promising strategy aimed at decreasing liver and kidney toxicity of cisplatin chemotherapy, while preserving the efficacy, provides for the use of Pt(IV) complexes as prodrugs, which can be intracellularly activated by reduction to Pt(II) species [111]. Indeed, longer life of Pt(IV) with respect to labile Pt(II) complexes can favour transport of larger amounts of intact prodrug for activation at the tumour site, avoiding side reactions with proteins and other biomolecules. Thus, Pt(IV) compounds can be expected to be less toxic and to produce fewer side effects *in vivo* [111].

Notably, functional groups on the axial ligands in Pt(IV) complexes provide sites for conjugation of the prodrug with targeting ligands or for imparting properties for optimal loading into delivery systems.

In an explorative study, the axial succinates of *cis*,*cis*,*trans*-[$Pt(NH_3)_2Cl_2(succinate)_2$] were mono- or bis-conjugated by amide linking to linear RGD or cyclic c(CRGDC) and c(RGDfK) integrin ligands (35–39 Fig. 8A) [112]. The constructs showed *in vitro* anti-proliferative effects on human endothelial and cancer cells. A hydrophobic Pt(IV)-analogue of cisplatin, [$Pt(hexanoate)_2$] 40 (Fig. 8B) was encapsulated in PLGA-block-PEG polymeric NPs 41 (Fig. 8B) labelled with the cRGDfK peptide [113]. *In vitro* evaluation in prostate (PC3 and DU145), and breast cancer (MCF7) cells and in their metastatic counterparts (PC3MLN4, MCF7MFP1, DU145LN2), expressing different levels of $\alpha_v\beta_3$ integrin, showed negligible differences in cytotoxicity between targeted and non-targeted NPs, although both formulations have enhanced cytotoxicity with respect to free cisplatin. *In vivo* evaluation of integrin targeted, Pt(IV)-loaded NPs in mice bearing MCF7 breast cancer showed tumour growth inhibition of ca. 60% (comparable with that observed with cisplatin) but a biodistribution profile suggestive of reduced nephrotoxicity. Other NP formulations carrying Pt(IV) prodrugs with biological investigations limited to *in vitro* experiments are discussed in Section 4.3 [114,115].

Other Pt(IV) prodrugs, well designed for *in vitro* studies, have afforded new tools exploiting activatable fluorophores for mechanistic studies of prodrug uptake and activation [116,117]. In the first study activation was dependent upon cleavage of caspase-sensitive DEVD peptide (42 Fig. 8C) [116]. In the second, reduction of Pt(IV)-disuccinate prompted the release of active Pt(II) as well as of succinate-linked DOX (43 Fig. 8C) [117]. In both studies activation of fluorescence indicated preferential uptake in $\alpha_v\beta_3$ expressing U87MG human glioblastoma cells.

Enhancement of activity and selectivity of the *cis*-ammine(2-methylpyridine)-dichloridoplatinum(II) (picoplatin) was sought by conjugation of its Pt(IV) prodrug, carrying a succinic axial group, with mono- and tetrameric integrin ligand [118]. Tetrameric c(RGDfK) (45, Fig. 8D), was obtained employing the regioselectively-addressable functionalised template (RAFT) scaffolds developed by Dumy's group [119]. This class of tetrameric RAFT-RGD has shown 10 times higher affinity for integrin $\alpha_v\beta_3$ than the monomeric ligand [120] and provided the possibility of linking other moieties, such as fluorescent dyes,

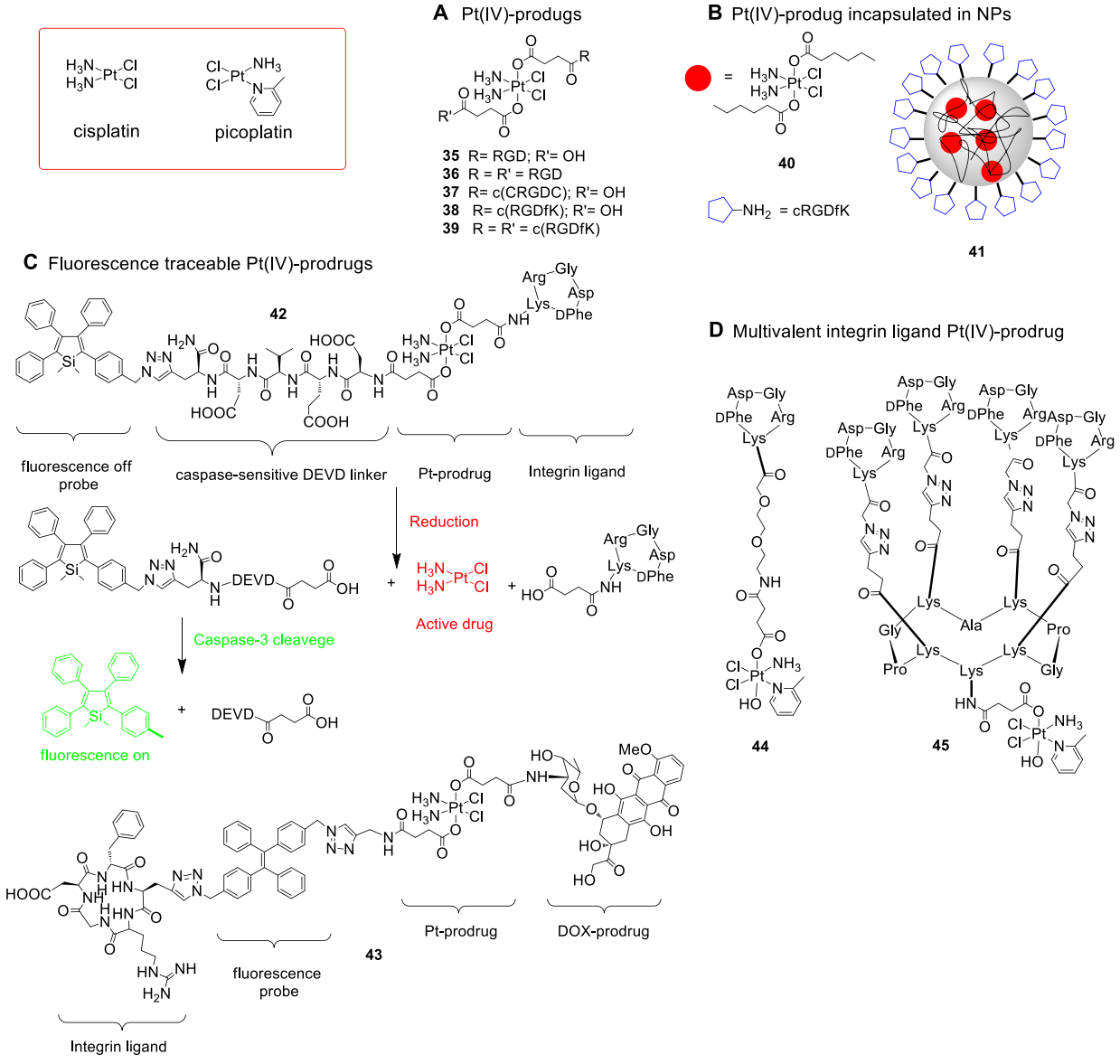

Fig. 8. A) Integrin targeted Pt(IV)-prodrugs [112]. B) Pt(IV)-prodrug in integrin targeted NPs formulation [113]. C) Activable-fluorescence Pt(IV)-prodrugs [116,117]. D) Multivalent Pt(IV)-prodrug [118].

radionuclide chelators for imaging [121,122] and drugs for targeted delivery [123,124]. Mono and tetrameric constructs 44 and 45 were tested for anti-proliferative activity in $\alpha_v\beta_3/\alpha_v\beta_5$-expressing melanoma SK-MEL-28 cells, and in CAPAN-1 and 1BR3G cell lines, both with low integrin expression. They showed enhanced activity in SK-MEL-28 cells with respect to picoplatin, with better IC_{50} values for the tetramer 45 (IC_{50} of 2.1 ± 0.8 μM vs. 18.5 ± 5.5 μM of 44), whereas no effects were shown in cells with low integrin expression. The activity correlated with intracellular accumulation of platinum (determined by ICP-MS), the latter increasing in proportion with both integrin expression and number of conjugated ligands. Although the overall results appear to depend mostly upon the picoplatin prodrug, it may be noted that tetrameric RAFT-RGD peptide showed some antiproliferative activities in melanoma cells, indicating interest in investigations of the properties of multivalent RGD-ligands [118].

3.5. Fumagillin prodrugs

Fumagillin, a mycotoxin produced by *Aspergillus fumigatus*, has been investigated as an anti-angiogenic agent in experimental models of cancer, arthritis and atherosclerosis. Lanza and co-workers, following prior studies in which they delivered fumagillin using a nanocarrier system targeting $\alpha_v\beta_3$ in angiogenesis [125–130], recently developed a lipase-labile fumagillin prodrug that improves retention in the circulation and is stable towards light. [131]. Introduction of a phospholipid moiety (Fig. 9) allowed incorporation into the phospholipid monolayer membrane of perfluorooctylbromide (PFOB) NPs. The NPs were additionally decorated with a quinolonic $\alpha_v\beta_3$-integrin ligand conjugated to PEG_{2000}-phosphatidylethanolamine (Fig. 9). According to the proposed mechanism, NPs interact with a target cell via integrin ligand, leading to the hemifusion of the two lipid membranes and resulting in a mixed monolayer that allows the transfer of the prodrug to the inner side of the cell membrane. At the site, local phospholipases can release the drug by enzymatic Sn 2-cleavage of the phospholipid moiety (Fig. 9). These nanoparticles were evaluated *in vitro*, and *in vivo* in a Matrigel plug model of angiogenesis in mice. MRI indicated decreased angiogenesis, with respect to control NPs (non targeted, or not loaded, or loaded with free fumagillin) [131]. *In vivo*, in KRN serum-induced arthritis in mice, ankle thickness and arthritis scoring were dose-dependently improved with respect to non-loaded NPs. [132]. Moreover, increased stability of the formulation allowed the lowering of effective dose to 0.3 mg/kg of prodrug from 2.5 mg/kg of free fumagillin [129,130].

4. Progress in integrin-targeted nano-delivery systems

NP carriers can improve efficacy and/or tolerability of drugs in clinical use or under investigation, modifying solubility, improving ability to trespass biological barriers, preventing off-target degradation and metabolisation, and overcoming mechanisms of drug resistance. Therapeutic and diagnostic NPs span a wide range of sizes (from 1 nm to over 200 nm) with different shapes (spheres, tubes, rods, dendrimers, etc.) and compositions (phospholipids, proteins, hydrophilic and lipophilic polymers, carbon, silica, metals, etc.) [133]. Hurdles encountered in the development of effective delivery systems, and ways for overcoming them, have been analysed [134]. Covering the surface of NP delivery systems with polyethylene glycols (PEGs, PEGylation) has offered significant progress, by improving solubility, and even more by providing a "stealth" effect, thus avoiding sequestration by the reticular endothelial system (RES). In this way, useful life in circulation could be prolonged, while preserving and enhancing permeability and retention in tissues, specifically of tumours, owing to peculiar properties of tumour vasculature (EPR effect) [29,30]. This "passive targeting" by PEGylation, as well as tissue uptake of albumins (see Section 4.5.4) has provided clinical opportunities, as proven by Abraxane™ (nab-PTX, i.e., nano albumin-bound, PTX-loaded particles) [135], Doxil/Caelyx™ (PEGylated liposomal doxorubicin) [136], and Myocet™ (liposomal DOX) [137].

The active targeting of tumour specific markers, and specifically of integrins, is pursued for implementing the intrinsic properties of the nanoparticles. The most investigated types of integrin targeted NPs (liposomes, polymeric nanoparticles, micelles, dendrimers, etc.), already reviewed by us and others [7,14,16], are still the mainstream of research, being employed in novel sophisticated developments which are discussed in other sections. In this section, we focus upon particles constituted of novel materials, or representing innovative modalities of delivery.

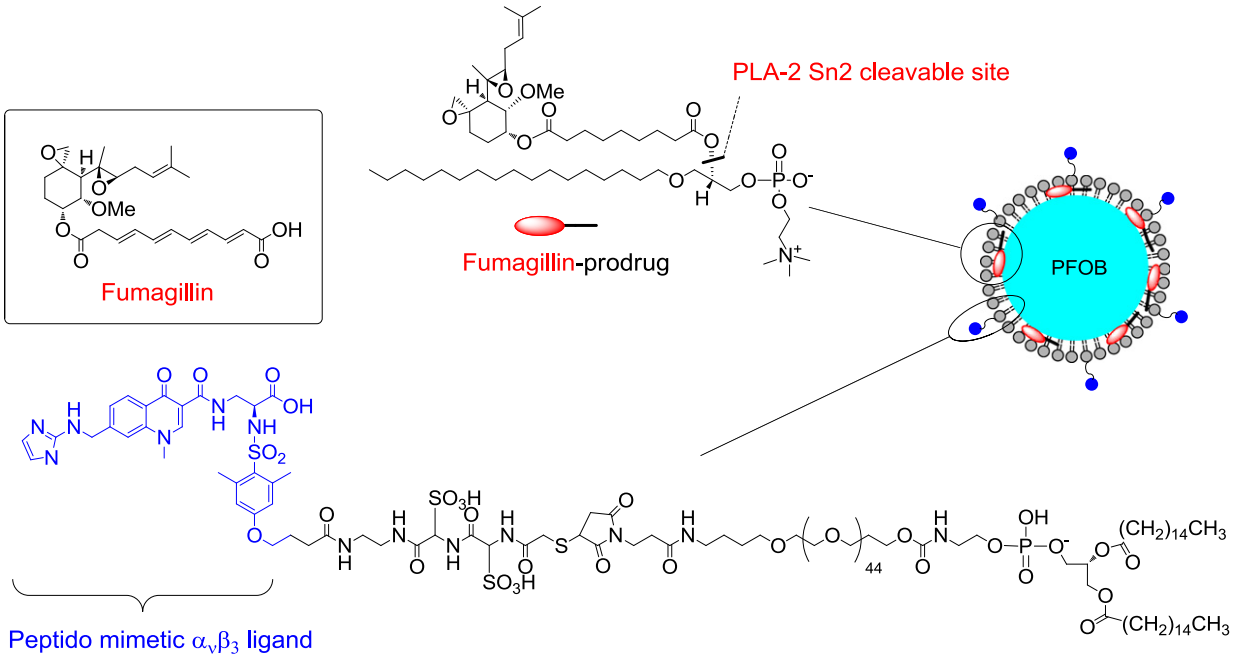

Fig. 9. Fumagillin prodrug in perfluorooctylbromide (PFOB) particles [131,132].

4.1. Graphene and graphene oxide nanoparticles

Graphene and graphene oxide (GO) nanoparticles, consisting of single layers of carbon atoms packed in a bidimensional (2D) honeycomb lattice [138], are of interest in drug delivery on account of their biocompatibility and their high surface area, suitable for loading of molecules by π–π stacking and van der Waals interactions, and also by chemical conjugation of GO [139,140]. Applications in photothermal-responsive tools for cancer treatment, owing to intrinsic high absorbance in the near-infrared (NIR) are also of interest.

Dai and co-workers [141] reported the first example of integrin-targeted GO–NPs. Nano-sized GO sheets (ca. 20 nm in planar dimensions) were linked to amphiphilic PEGylated polymer chains for improving solubility, and then conjugated via amino terminals of the PEG chains to the NIR dye Cy5 and to c(RGDfC). The construct exhibited selective uptake in U87MG cancer cells and provided effective photoablation of these cells *in vitro*, without cytotoxicity in absence of NIR irradiation.

Wang *et al.* used c(RGDfK)-modified chitosan for coating GO–NPs loaded with DOX (Fig. 10A) [142]. The system was characterised by efficient loading of DOX (1 mg/mg of GO) and pH-responsiveness for drug release, owing to loosening of hydrogen bonding of GO and chitosan at low pH (5.5). *In vitro* uptake, with consequent reduced proliferation, in hepatoma cells (Bel-7402, SMMC-7721, HepG2) proved the affinity for $\alpha_v\beta_3$-integrin overexpressing cells.

A nanosystem, with positively charged surface, prepared by coating GO with polyethyleneimine (PEI), was coupled with a monoclonal-antibody (mAb) selective for $\alpha_v\beta_3$ integrin [143]. An anionic polyelectrolyte, prepared by reacting citraconic anhydride with poly(allylamine) (PAH-Cit) and then conjugated with DOX, was loaded by ionic interaction on the positively charged system (Fig. 10B). Citraconic conjugation is known to release DOX in mildly acidic conditions. Selective transport of DOX into integrin over-expressing U87MG glioblastoma cells was assessed by fluorescence and cytotoxicity assays. Prolonged half-time in blood circulation (2.1 h) for these mAb-NPs, in comparison with free DOX (0.52 h), and enhanced tumour uptake 6 h after administration in U87MG tumour-bearing nude mice were ascertained by *in vivo* biodistribution studies. In histological assays, the NPs appeared to be devoid of liver and lung toxicity, notwithstanding the high RES uptake [143].

Nanodiamonds, another nano-structured carbon material with biomedical applications [144], are now employed in integrin-targeted delivery systems (Section 2.5) [85].

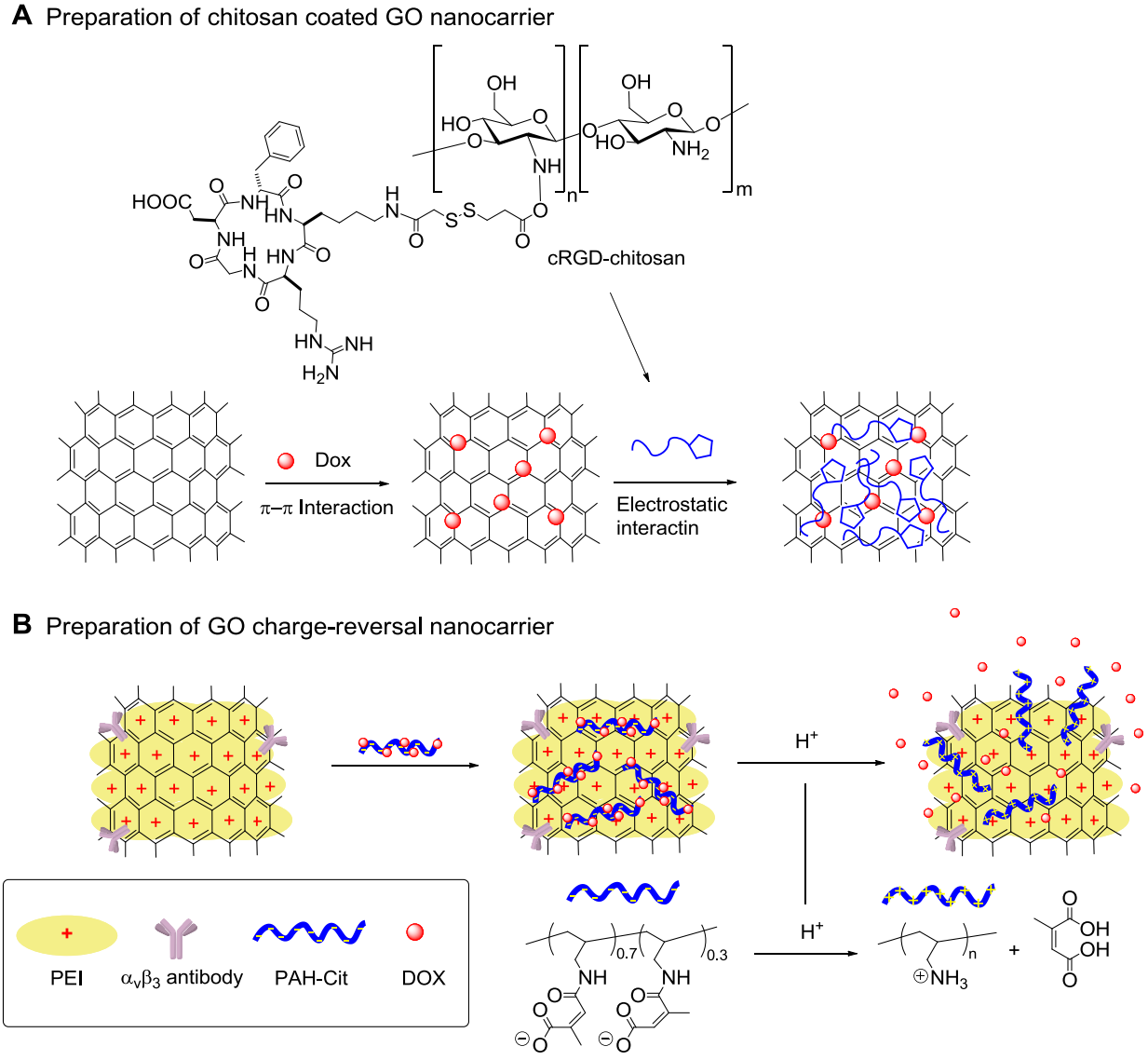

Fig. 10. Preparation of: A) GO nanocarriers loaded with DOX and linked to cRGD-modified chitosan [142] and B) pH-responsive GO nanocarriers coated with polyethyleneimine (PEI), coupled with an $\alpha_v\beta_3$ integrin mAb, and loaded with DOX-linked anionic carboxylate polyelectrolyte PAH-Cit [142].

4.2. Mesoporous silica nanoparticles

Nanoparticles constituted of mesoporous silica (MS-NP) [145], arouse increasing interest for drug delivery, because of their large surface area and volume of pores, which afford marked capacity of drug loading, and possibility of surface conjugation with drugs or targeting labels. [146–148]. Low toxicity may confer advantage over other materials.

Ferris *et al.* prepared fluorescein-labelled MS-NPs, linked through disulphide bonds with integrin ligand cRGDfC and loaded with CPT [149]. The NPs selectively targeted metastatic cancer cells (MDA-MB 435), affording 10-fold enhancement of uptake and increased cytotoxicity with respect to free drug and to non-targeted NPs.

Pan *et al.* prepared 3-aminopropyl triethoxysilane-coated MS-NPs dually labelled with internalising peptide TAT (see Section 5.1) and integrin ligand cRGDyC [150]. The particles were loaded with DOX. Sequential targeting of cellular membranes and nuclei was evaluated *in vitro* in human cervical cancer HeLa cells with high $\alpha_v\beta_3$ expression and in human embryonic kidney 293T cells (low $\alpha_v\beta_3$ integrin expression); showing enhanced nuclear accumulation of DOX in HeLa cells in comparison with 293T cells. *In vivo* evaluation in nude Bal/bc mice, implanted subcutaneously with HeLa cells, showed accumulation of RGD/TAT particles in tumours at 24 h post-injection (ca. 8.7% of injected dose (ID)/g), markedly more than non-labelled particles and also more than particles carrying only TAT (ca. 1.05 and 4.6% ID/g, respectively). Accumulation correlated with tumour regression.

Aiming at MMP mediated release, Zhang *et al.* exploited a multifunctional coating of a DOX-loaded MS-NP, by linking β-cyclodextrin (β-CD) through a disulphide bridge, loading a peptide combining RGD and matrix MMP-sensitive sequences, i.e., Pro-Leu-Gly-Val-Arg (PLGVR), in the (β-CD) moiety, and conjugating poly(aspartic acid) (PASP) at the N-terminal of the peptide (Fig. 11A) [151]. The particles were incubated with SCC-7 (squamous cell carcinoma) cells and HT-29 (human colon cancer) cells, expressing high levels of MMPs, in order to evaluate targeting and triggered drug release ability. The fluorescence of released DOX was observed after 4 h incubation in both cell lines, but not when an MMP inhibitor was added. The results were consistent with those of PASP release curves and with cytotoxicity. The latter was shown to be inhibited by the MMP inhibitor (over 70% cell viability vs. 40% without inhibitor). The increase of DOX fluorescence in SCC-7 and HT-29 cells (1.7-fold and 1.4-fold respectively) with RGD-labelled NPs with respect to NPs carrying a non-RGD sequence was observed.

Chen *et al.* prepared luminescent MS-NPs for both imaging and drug delivery [152]. Luminescence was imparted to the particles, covered with 3-aminopropyltriethoxysilane (APTES), by calcination at 400 °C, generating small carbonaceous dots. The particles were labelled with c(RGDyK) and loaded with DOX. Efficient internalisation in U87MG cells was observed in comparison with non-targeted NPs. In mice with U87MG subcutaneous grafts, luminescence assays indicated increased accumulation in tumours at 4 and 24 h with respect to non-targeted NPs. Tumour accumulation of RGD labelled NPs was confirmed by histological examination of excised specimens, revealing marked fluorescence of carbonaceous dots and DOX, but not in the case of unlabelled NPs. The observation that fluorescence signals correlated with shape of blood vessels suggested that most of the RGD-labelled NPs were retained in the blood vessels by interaction with integrin $\alpha_v\beta_3$.

Coating of MS-NPs with synthetic or natural polymers can improve solubility, avoid non-specific adsorption of proteins, as well as provide functional groups for conjugation. Zhu *et al.* envisaged the use of poly(carboxybetaine)-chains carrying catecholic DOPA moieties for imparting adhesive properties on silica, to protect MS-NPs from interactions with plasma proteins. The coating also allowed conjugation with the integrin ligand cRGDyK at the carboxyl terminal, favouring uptake in endothelial cells [153].

Liao *et al.* incorporated DOX-loaded MS-NPs into microspheres of the natural polysaccharide alginate [154,155]. The coating conferred excellent biocompatibility and sustained release properties, and offered conjugation of its carboxyl groups with linear peptide KYRGD. Cell viability assays and confocal laser scanning microscopy (CLSM) indicated that the microspheres targeted and internalised DOX into BT-20 breast cancer cells [154] and HepG2 human liver cancer cells [155], resulting in both cases in enhanced cytotoxicity in comparison with non-targeted particles.

Porous silicon nanoparticles (PSi), obtained by electrochemically etching of monocrystalline silicon wafers, and functionalised with amino groups by APTES, were used for integrin targeting [156].

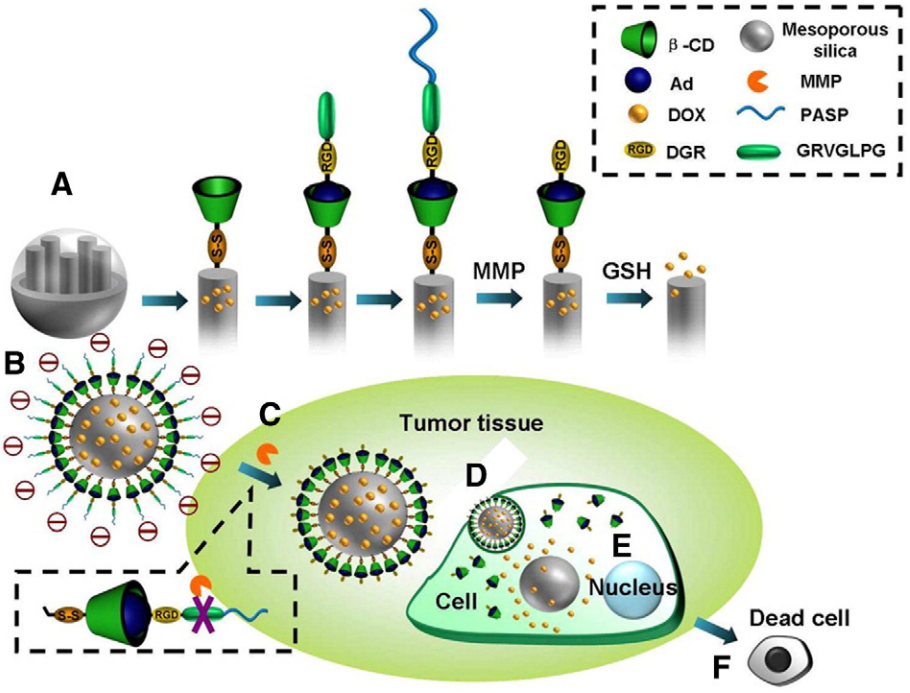

Fig. 11. Schematic representation of sequential steps A–F of intracellular drug release by MS-NP. Reprinted with permission from ref. [151]. Copyright (2013) American Chemical Society.

Surface labelling, either with RGDS or the cell-penetrating iRGD [104] (see Section 5.2), both modified with azide functions, was achieved by click-coupling. The particles were then loaded with sorafenib and were tested on proliferation of EA.hy926 endothelial cells, with effect similar to that of the free sorafenib in DMSO solution.

4.3. Silica coated NPs

Coating with amorphous silica can be employed to stabilise various NPs and provide a surface suitable for introducing functions for conjugation. Pt(IV)-containing NPs of formula $Tb_2(DSCP)_3(H_2O)_{12}$, obtained by co-precipitation of DSCP (*c*,*c*,*t*-(diammine-dichloro-disuccinato)Pt(IV)) and Tb^{3+} ions, were stabilised by silica coating, prolonging release of Pt species up to 9 h [114]. Integrin targeting was achieved by grafting a derivative of c(RGDfK) on the silica surface. *In vitro* evaluation in the human colon carcinoma cell line HT-29, expressing $\alpha_v\beta_3$, integrin, showed enhanced cytotoxicity with respect to free cisplatin and to non-targeted particles. Thus, internalised particles presumably released the DSCP moieties, with reductive conversion into effective Pt(II) species (see Section 3.4). It appears that these NP formulations could allow the design of effective delivery vehicles for a variety of therapeutic or diagnostic purposes.

A theranostic approach was explored by Taylor-Pashow *et al.* with silica coated NPs combining MRI imaging with Pt(IV) delivery [115]. Hybrid metal–organic nanoparticles were synthesised by heating an equimolar solution of $FeCl_3$ and terephthalic acid, also exposing amino groups by incorporation of 2-aminoterephthalic acid. This allowed the conjugation of ethoxysuccinato-cisplatin (i.e., *c*,*c*,*t*-$[PtCl_2(NH_3)_2(OEt)(O_2CCH_2CH_2CO_2H)]$). As in the previous study [114], the construct was coated with a thin shell of silica and labelled with a silyl derivative of c(RGDfK). *In vitro* evaluation on the HT-29 cell line showed cytotoxicity comparable to the free drug, but better than untargeted NPs.

4.4. Chitosan nanoparticles

Polysaccharides are widely employed as natural biopolymers with advantages over synthetic polymers, such as easy availability, presence of functional groups for conjugation, biocompatibility and biodegradability. Among them, chitosan has found a broad application in the design of biomaterials for tissue regeneration and in drug delivery, owing to the amino groups exposed on its backbone. These impart cationic properties and are also suitable for conjugation with drugs, targeting ligands, imaging dyes, etc. Insertion of integrin ligands has been explored in tissue engineering, in the aim of simulating the ECM environment and favouring adhesion of integrin-expressing cells [157–160]. In drug delivery, biocompatible chitosan is a good basis for formulation of polymeric NPs and for coating and targeting of GO–NPs [142]. It can also improve the biodistribution properties of inorganic nanoparticle.

The integrin ligand cRGDyK was encapsulated in chitosan-based and 5β-cholanic acid modified NPs, as an antiangiogenetic agent with sustained release in tumour vascularisation [161]. Its intratumoural administration decreased microvessel formation and tumour growth with respect to the free peptide. Labelling of chitosan polymers with integrin ligands has also been adopted for targeting. Cai *et al.* conjugated the amino groups of chitosan with stearic acid and PEG [162], thus generating an amphiphilic block-copolymer able to self-aggregate into spherical micelles in aqueous media [163]. A linear RGD peptide was also linked through a bifunctional PEG. The micelles were loaded with high efficiency with DOX and were tested *in vitro* in integrin-overexpressing human hepatocellular carcinoma cells (BEL-7402) and in human epithelial carcinoma cells (Hela). The RGD-labelled micelles significantly increased DOX concentration in BEL-7402 cells with respect to non-targeted micelles, whereas no difference was observed in Hela cells. *In vitro* cytotoxicity assays in BEL-7402 cells confirmed the enhanced efficacy of the RGD-modified micelles, also in comparison with the free drug.

Ma and co-workers used O-carboxymethyl-chitosan nanoparticles for improving the delivery of insoluble PTX [164]. An oil–water–oil double emulsion method was combined with a temperature programmed solidification technique in order to achieve a high loading efficiency of the lipophilic drug within the polar chitosan matrix. The loaded NPs were further modified by conjugation of PEG chains carrying the integrin ligand cRGDfC on one terminal. The labelling enhanced internalisation in Lewis lung carcinoma (LLC) cells with respect to non-labelled NPs, with increased cytotoxicity. Improved stealth effect of PEGylated NPs, with respect to the non-PEGylated ones, was attested by their lower uptake in J774A.1 macrophages. *In vivo* biodistribution studies in LLC-bearing mice, RGD functionalised chitosan NPs loaded with the dye DIR (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine) showed markedly increased fluorescence at tumour site up to 48 h. In treatment of LLC-bearing mice with PTX loaded NPs, tumour growth was significantly inhibited by RGD-labelled NPs with respect to both non-targeted NPs and free drug. Interestingly, the authors noted rapid tumour growth in the PTX group of animals at the end of the treatment, at difference from the group treated with RGD-labelled NPs, suggesting that the latter may accumulate at tumour sites providing sustained drug release.

The cationic properties of chitosan NPs were exploited in the delivery of oligonucleotides, as exemplified by Han *et al.* with a cRGDfK-labelled chitosan in the delivery of siRNAs silencing different growth-promoting genes (POSTN, FAK and PLXDC1) in ovarian cancer cells (Section 9.1) [165].

4.5. Biomimetic, or "bioinspired", delivery systems

Various research groups seek inspiration in nature for overcoming some limitations of classical delivery systems. The way in which complex proteins, or other molecular building blocks, assemble or disassemble, and the mechanisms of their transport in tissues, can suggest new modalities for the design of innovative delivery systems [166].

4.5.1. Ferritin

A good example is provided by ferritin, representing a "bioinspired nanosystem" exploitable for biotechnological applications. Its cage structure offers room for high load of a variety of molecules, including metal ions and complexes, for diagnostic, therapeutic, and theranostic systems [167]. Applications in integrin-targeted delivery [41,42] exploited previous experience with ferritin genetically modified to include the RGD4C moiety on the exterior surface, and then loaded with Fe_3O_4 nanoparticles for imaging of C32 melanoma cells *in vitro* [168]. The experience was extended in the use of RGD4C-ferritin in bimodal imaging *in vitro* and *in vivo* with ^{64}Cu for PET and Cy5.5 for NIR [169]. The NIR probe was chemically conjugated on normal ferritin. Then Cy5.5-ferritin and RGD4C-ferritin were mixed and brought to pH 2, thus destructuring the ferritin cages in subunits, which reassembled chimerically at pH 7.4 (Fig. 12). *In vivo* in U87MG glioma xenografts in mice, PET and NIR showed at 24 h accumulation in tumours, confirmed by *ex vivo* PET on tumour specimens.

Use in delivery was at first aimed at chemotherapy with a DOX-copper(II) complex [42], and then at photodynamic therapy with the photosensitiser zinc hexadecafluorophthalocyanine (ZnF16Pc) [41]. The DOX-copper complex exhibited markedly enhanced loading with respect to free DOX. Following various *in vitro* and *in vivo* experiments, an efficacy study was performed in mice with subcutaneous U87MG glioblastoma implants. After 5 i.v. injections in 2 weeks and 18-day follow-up, treated animal showed 89% inhibition of tumour growth with respect to control animals, against 74% showed by free DOX at the same dose (5 mg/kg). Caspase-3 staining, taken as an expression

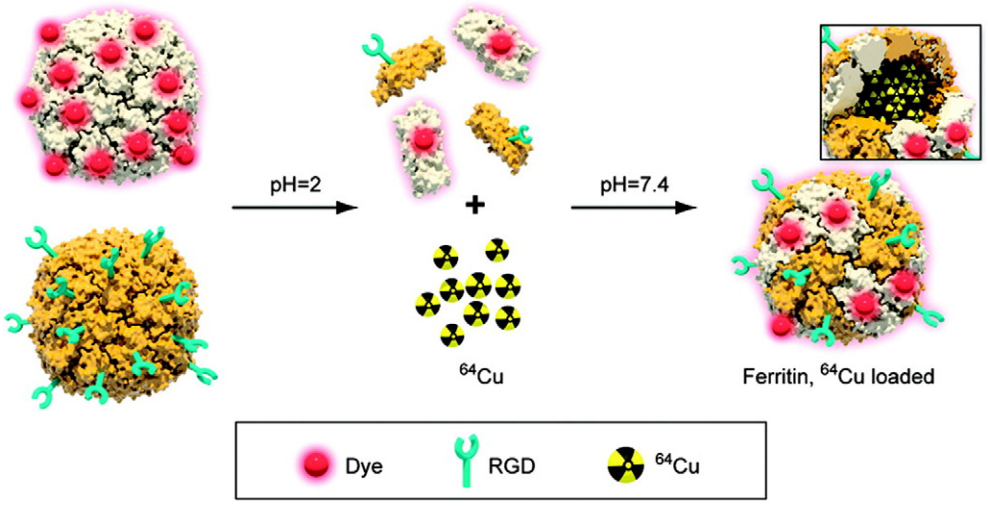

Fig. 12. Schematic illustration of chimeric cages assembly. Reprinted with permission from ref. [169]. Copyright (2011) American Chemical Society.

of cardiotoxicity, appeared markedly lower in the ferritin-complex treated animals.

In the second study, ZnF16Pc was incorporated at 1.5 mg/mg of RGD4C-ferritin [41] affording small particles (19 nm) with high load, and high tumour accumulation at 24 h (27-fold with respect to normal tissues) in subcutaneous U87MG tumours in mice. Photoirradiation at 24 h post administration induced marked tumour inhibition (83.64% after 12 days), with minimal toxicity in skin and major organs. Altogether, these studies indicate the capability of an integrin targeted ferritin cage structure to serve as a nanoscale container for tumour imaging and effective drug delivery.

4.5.2. Peptidic nanofibres

The self-assembling of proteins in specific tridimensional structures is a peculiar process occurring *in vivo*, regulating their stability and biological activity. Several biomacromolecules (e.g. antibodies, collagen, or viral capsides) perform their function by appropriate assembling.

Self-assembling nanofibres of synthetic peptides attracted considerable interest for easiness of preparation and good biocompatibility and have been used in tridimensional cell cultures, in tissue engineering and in regeneration [170], as well as in drug delivery for enhancing solubility and accumulation in tumours. Encapsulation of CPT in amphiphilic peptide fibres improved antitumour efficacy *in vitro* and *in vivo* in a model of human breast cancer [171]. Nanofibres constituted of the cell-penetrating peptide TAT favoured endocytotic uptake of PTX-loaded NPs [172].

Liu *et al.* reported the integrin targeting of a synthetic self-assembling nanofibre, based on the peptide naphthalene-GFFYG-RGD [173]. The peptide, loaded with curcumin during assembly, was tested in $\alpha_v\beta_3$-integrin expressing HepG2 liver carcinoma cells showing significantly higher uptake of curcumin, with consequent higher cytotoxicity in comparison with similar nanofibres carrying the RGE sequence. *Ex vivo* experiments, following i.v. administration of targeted nanofibres, indicated marked accumulation of curcumin in tumours of mice with HepG2 xenografts.

Genetically modified collagen-like protein, expressed in *Escherichia coli* and capable of auto-assembly in a stable triple helix, can be used in preparation of hydrogels. An integrin labelled collagen-like protein was obtained by insertion of the GFPGER sequence [174,175], recognised by $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins as well as by $\alpha_{10}\beta_1$ and $\alpha_{11}\beta_1$ [176], was PEGylated and functionalised with photo-crosslinkable acrylate-PEG-NHS (Acr-PEG-NHS), preserving, although somewhat decreased, availability of integrin binding sites for adhesion of bovine endothelial cells. These preliminary results suggest potential developments in biomaterials applicable in tissue regeneration and drug delivery.

4.5.3. Particle targeting with endogenous proteins

Caracciolo *et al.* explored a new way of labelling NPs by attracting a soluble ECM protein to their surface, thus imparting affinity for integrin-expressing cells [177]. Lipid particles of cationic 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and Cy3-fluorescent DNA ("lipoplexes") spontaneously assembled in aqueous solution. These particles, when incubated in human plasma, were spontaneously covered by vitronectin. *In vitro* experiments showed that plasma-incubated particles were taken up by highly metastatic MDA-MB-435S cells overexpressing integrin $\alpha_v\beta_3$. The novelty of this approach, involving the ability of nanoparticle of attracting *in vitro*, or *in vivo*, a cover of proteins ("protein corona effect") [178], could open an alternative way in the design of targeted delivery systems.

4.5.4. Albumin nanoparticles

Human or bovine albumins have been widely exploited in the development of prodrugs and delivery systems, on account of their known favourable properties of uptake in tissues, specifically in tumours, and at sites of inflammation [179–181]. Indeed, PTX-loaded albumin NPs (Abraxane™) have been approved for the treatment of metastatic breast cancer [135]. In order to target albumin- and albumin coated-NPs for tumour cell uptake and accumulation, their labelling with known and novel integrin ligands has been investigated [55,182]. Labelling with cell-penetrating peptides is proposed as well, with promising results [104,183,184] (see Sections 5.2 and 6.4). Technological progress in self-assembling particles obtained by direct mixing of PTX and albumin conjugated with RGD labels and theranostic moieties is reported (Section 7.4) [185].

4.5.5. Targeted delivery of cell organelles

An *in vitro* study of flow cytometry and confocal microscopy was conducted on novel nano-sized vesicles (exosomes). These were derived from cultures of mouse immature dendritic cells [186], and were loaded with DOX by electroporation. The study showed efficient targeting in breast cancer cells, which was confirmed by inhibition of tumour growth, without apparent toxicity, in tumour models *in vivo*.

4.5.6. Targeted delivery of a cell

A peculiar situation, in some way representing a reversal of usual approaches, is represented by integrin-targeting of a cell, intrinsically endowed with immunological potential, via genetic modification. Fu

et al. manipulated T-cells, as cancer cell killers, by engrafting a DNA sequence encoding a modified echistatin (28-Met was replaced with Leu, to reduce $\alpha_5\beta_1$ affinity), in order to specifically target $\alpha_v\beta_3$ expressed in tumour vessels [187]. Killer properties were ascertained in HUVECs and in $\alpha_v\beta_3$-expressing cancer cells. *In vivo* these T-cells caused bleeding in tumour tissue and shrinkage of tumour in mice implanted with B16-F0 murine melanoma. Specific tumour permeation and uptake of rhodamine-labelled liposomes were also reported, indicating the potential of co-therapy with drug-loaded nanoparticle.

4.5.7. Phage-derived particles

Chen and co-workers reported a viral-based delivery system using an intact T7 phage as a scaffold for multiple copies of a peptide carrying Cu-binding hexahistidine motifs and RGD4C integrin ligands [188]. The recombinant phage was able to load Cu(II) ions, whose reduction to Cu(0) resulted in a very stable hybrid phage. It retained copper and preserved structural integrity and target specificity. These hybrid particles were internalised by integrin expressing MCF-7 breast cancer cells, against no uptake in normal cells (MCF-12F). Thus, a possible use in a ^{64}Cu-PET probe is envisaged.

5. Cell penetrating peptides

Anticancer agents almost generally act at intracellular sites. Thus, after recognition by cellular membrane receptors, the integrin-targeted system or at least the active agent deprived of the targeting moiety should penetrate into the cell, in case after removal of PEG chains or of other features employed for appropriate biodistribution.

5.1. RGD and TAT peptides

Useful tools to the purpose were first offered almost 30 years ago by cell penetrating peptides, starting from the discovery of TAT (i.e., the dodecapetide GRKKRRQRRRPQ, named from TransActivation of Transcription of HIV). The properties of this peptide and others with similar properties prompted since 1997 their application in delivery systems [189]. Thus, various cell penetrating peptides, either naturally available, chemically modified, or fully synthesised, were applied for delivering of proteins, genes, nucleotide sequences such as siRNA, mixed peptides–oligonucleotides, as well as nanoparticles and small molecules [190, 191]. Different techniques were applied, involving either covalent binding or complex formation between the peptides and their "cargo" [190, 191].

Use in integrin-targeting was only recently started. Two studies using the TAT peptide were reported by Xiong *et al.* [37,38]. In the first they devised a carrier for siRNA based on poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-PCL), labelled with the RGD4C peptide and with TAT on the external shell, and added with polycationic spermine in the core for siRNA binding. This latter was aimed at overcoming drug resistance via the P-glycoprotein mechanism. Indeed, in DOX-resistant MDA435/LCC6 cell lines the construct reversed the resistance, reinstating accumulation of DOX. Dual RGD/TAT-micelles were more effective than mono-decorated ones [37]. In a second study, the micelles were also conjugated with DOX via a pH-sensitive hydrazone link. Improved delivery of both drugs was demonstrated in multidrug-resistant MDA-MB-435 human tumour, first in cells *in vitro* and then in a mouse model *in vivo* [38].

A recent study reported the application of both TAT and RGD to liposomes carrying a NIR probe. The NPs were also modified through disulphide bond with PEG_{5000}, in order to increase the circulation time [192]. The reductive cleavage of the bond (see Section 6.3) by a dose of L-cysteine allowed the exposure of the hidden ligands directly in tumour tissues. Following the internalisation *in vitro* in human hepatoma (HepG2), human cervical carcinoma (HeLa) cells, and in HepG2 spheroids, *in vivo* as well as *ex vivo* NIR-imaging experiments in tumour specimens, support the "tremendous potential of these multistage liposomes". Confirmation of the efficacy of NPs loaded with active drugs is expected.

The combination of TAT and RGD-labelling on mesoporous silica nanoparticles for DOX delivery has also been reported (Section 4.2) [150]. Biodistribution experiments indicate accumulation and retention up to 48 h in HeLa cell subcutaneously implanted tumours, with penetration of DOX and tumour regression.

5.2. RGD and iRGD peptides

Synthetic cell-penetrating peptides with RGD motifs in their sequence [193], some also including acid-sensitive amphiphilic sequences [194], are reported. Mokhtarieh *et al.* designed peptides suitable to adopt an amphipathic alpha-helical structure (e.g. RLLRLLRRLLRLLRR LLRC) for endosomal escape. They also added multiple RGD motifs (e.g. RGDRGDRRDLRLDRGDLRC) to induce integrin-mediated endocytosis and modified some sequences to increase basicity and lipophilicity (e.g. RGDRLDRRDLRLDRRDLRC) [193]. Control peptides were obtained via aspartic to glutamic shift in the RGD motif. The peptides were conjugated to "asymmetric" liposomes with 250 nm size, which suitably incorporate siRNAs [195]. The liposomes efficiently internalised siRNAs in NSCLC cells (A549, NCI-H322 and NCI-H460) and NIH-3T3 cells, thus supporting the usefulness of the peptides. Still, unexpectedly, cell penetration was observed regardless of the presence of integrin-targeting RGD motifs.

Of major interest is iRGD (internalising RGD), a peptide intrinsically endowed with both RGD-label and penetrating properties [104]. It consists of a cysteine-bridged peptide sequence (i.e., CRGDKGDPDC) discovered by the group of Ruoslahti [104] in a phage-displayed library, along with the active variants CRGDKGPDC, CRGDRGPDC, and CRGD KGPEC, having Arg in lieu of Lys and/or Glu in lieu of Asp-9. It was shown to home to tumours in a sequential process, at first binding at integrin $\alpha_v\beta_3$ by the RGD motif. Proteolytic cleavage leads to exposure of the RGDK motif that through the binding to the neuropilin-1 receptor mediates cell penetration (Fig. 13A). The RGDK/R motif of iRGD exhibits common features with the arginine/lysine-rich C-terminal of neuropilin-1-seeking peptides [196]. The study also showed that iRGD labelling of albumin-coated paclitaxel nanoparticles (Nab-PTX, or Abraxane™, in clinical use for metastatic breast cancer) enhanced by 10-fold their tumour accumulation in mouse models of human prostate and breast cancers. Furthermore, it was also shown [104] that iRGD can enhance the effects and improve the tolerability of anticancer drugs (DOX, DOX-liposomes, Nab-PTX, and trastuzumab) in various mouse models even when co-administered, without conjugation, exerting a so-called "bystander" effect. In the case of Nab-PTX, accumulation was 12-fold increased. The bystander effect of co-administration in models of glioblastoma also enhanced the *in vivo* effects of nanoparticles endowed with theranostic potential (see Section 8) [197–199]. It appears probable that the bystander effect may depend upon improved internalisation in tumour cells, and not just in vessels. It was suggested that the results encourage development towards clinical testing in glioblastoma and in other cancers.

The same group has shown that adding a cysteine on iRGD considerably increases its plasma life, and improves the accumulation in tumours [183]. This behaviour is mediated by a spontaneous conjugation by a disulphide link to plasma albumin, thus reducing renal excretion and enhancing extravasation of the conjugate. It may be incidentally noted that addition of cysteine to the C-terminal of a peptide, inducing albumin conjugation and protecting from protease degradation, has been experimented on glucagon-like peptide-1 [200], extending the reduction of blood glucose in mice from 1 h to 6 h. In case of iRGD, the modifications were aimed at both prolonging plasmatic life, and targeting albumin for tumour uptake. The well-known phenomenon of tissue uptake of albumin, specifically in tumours and sites of inflammation, has been exploited in the development of prodrugs and delivery systems [179–181]. Two iRGDs,

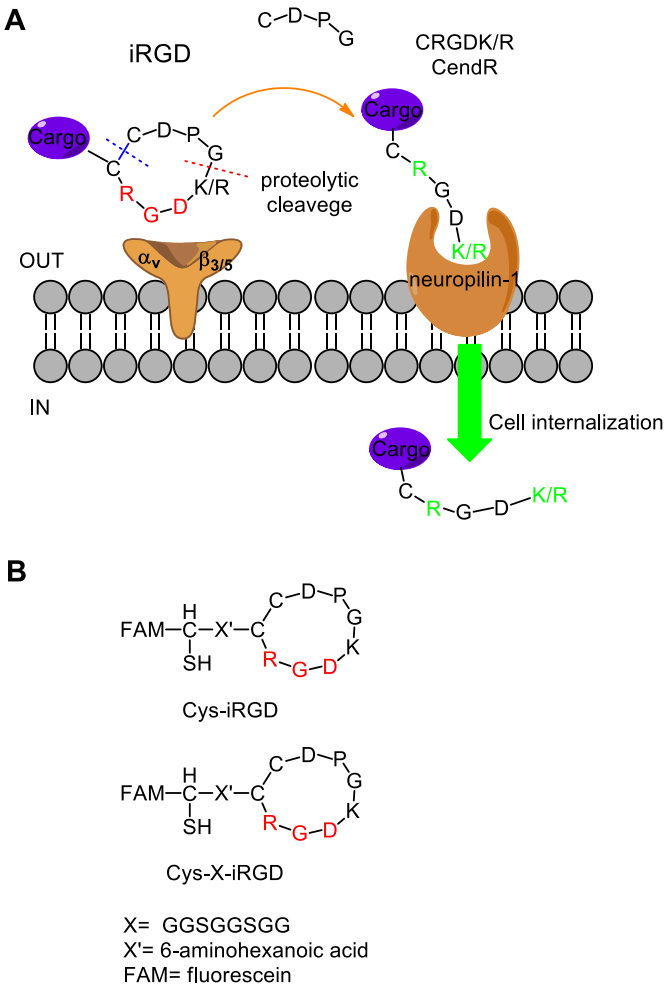

Fig. 13. A) Proposed mechanism of cell penetrating peptide iRGD [104]. B) Cysteine enriched iRGD variants [183].

modified with cysteine at the N-terminal (Fig. 13B), were used for albumin conjugation in *in vitro* and *in vivo* experiments of distribution and tumour uptake. In other experiments, the fluorescence quencher dabcyl was added at the C-terminal, in order to ascertain resistance to disulphide cleavage in plasma, which would cause restoration of fluorescence. *In vivo* experiments in mice bearing 4T1 murine breast cancer in co-administration with trastuzumab and Nab-PTX demonstrated substantially enhanced drug accumulation in tumours via bystander effect, with the elongated Cys-X-iRGD but not with Cys-iRGD (Fig. 12B) [183]. It was suggested that *in vitro* conjugation of Cys-iRGD to albumin could be exploited in albumin-based delivery systems (reviewed in [179]), improving both targeting and penetration. Furthermore, the evidence of *in vivo* conjugation suggests that coupling with cysteine might improve pharmacokinetics of other peptidic drugs.

The active domain (62 amino acids) of Bit1, a proapoptotic mitochondrial protein (179 amino acids) associated with cell anoikis, was engineered in a recombinant fusion with iRGD, which afforded 77% reduction of tumour volume by repeated intratumour injection in mice with orthotopically implanted breast tumours [201].

Investigation of cell penetration of iRGD-labelled DOX-liposomes, in comparison with non-labelled particles, in B16 melanoma cells, showed enhanced uptake and increased cytotoxicity [202]. Importantly, the results were supported by *in vivo* experiments in a B16 melanoma model.

Authors of the School of Pharmaceutical Science in Beijing investigated two iRGD labelled PEGylated liposomes, respectively loaded with DOX [203] or linoleic-conjugated PTX [204] in murine B16F10 cells and in melanoma-bearing mice. Both systems demonstrate superior *in vitro* targeting and penetration, as well as *in vivo* efficacy, in comparison with non-iRGD liposomes.

iRGD has also improved the delivery of acid sensitive *cis*-aconityl linked DOX in gliomas (see Section 8) [93].

5.3. iNGR peptide

The penetrating peptide, CRNGRGPDC (denominated iNGR) was synthesised by combining CRNGR, i.e., a neuropilin-seeking sequence with the amino peptidase N (CD13) directed NGR sequence, [205]. iNGR penetrated into tumour tissues more effectively than NGR peptide. The tumour-homing properties of NGR, first described in 1998 [17], its conjugation in delivery systems, and its combination in fusion protein with TNF, with resulting enhancements of penetration and efficacy, have been reviewed [206,207].

5.4. "Hunting–killing" peptides

An all-dextro peptide was used to avoid proteolysis and promote internalisation, using the HKP (Hunting–killing-peptide) approach of Arap, Pasqualini, Ruoslahti *et al.* [208]. Both NGR and RGD sequences were used as "hunting" moieties, while "killing" moieties were identified in peptide series able to disrupt mitochondrial membrane *in vitro*. The HPK-2 peptide (NH_2-ACDCRGDCFC-GG-$_{dextro}$(KLAKLAK)$_2$-COOH), obtained by conjugation of one of the peptides in full dextro sequence with a cyclic RGD peptide via a two glycines linker, showed inhibition of angiogenesis in a murine model of joint arthritis. The related HPK-1, including a CNGR sequence, showed anticancer effects in mice bearing MDA-MB-435 human breast carcinoma xenografts.

6. Induction of drug release by biochemical factors

Delivery systems combining the targeting of cancer cells with drug release mechanisms induced both by biochemical factors peculiar of cancer tissues (pH, enzymes, redox gradients), or by external stimuli (heat, ultrasound, magnetic field, visible light, ultraviolet or near infrared radiation) are in an early stage of investigation. The real value of this dual approach is critically discussed, considering the complexity of the simultaneous control of different factors, including those generally relevant in the biodistribution of particulate systems. Hurdles of technological feasibility may endanger the possibility of development [209,210].

6.1. Acidity of tumour tissues

Enhancement of drug release in the acid environment of cancer tissue, varying from 6.8 in the stroma to 5–6 in intracellular organelles (reviewed in [91,211,212]), has been investigated with particles obtained by the self-assembling of block copolymers with anionic and cationic chains, or by simple mixing of individual polymers. These particles, stable in neutral conditions, are prone to disassembly and release any loaded drug when their functions become protonated. Experience was gained with various pH-responsive polymers. Poly-L-lysine and polyethylimine (PEI) were investigated in nanosystems for the response to low pH of endosomes and lysosomes [211,212]. Easily accessible, soluble, bio-compatible and -degradable poly(L-glutamic acid) appears as the most useful anionic polymer for these purposes. Experience of conjugation with anticancer drugs and imaging agents, also with multiple or multimodal modification of a single polymer chain provided prodrugs of therapeutic and diagnostic interest, e.g. DOX, daunorubicin, 1-β-D-arabinofuranosyl-cytosine (Ara-C), cyclophosphamide, and melphalan. Advanced clinical experience with a PTX conjugate could be nearing approval for therapeutic use [213].

Micelles constituted by a single amphiphilic peptide including the RGD sequence were recently provided [194]. A synthetic 13-mer

peptide, joining C-terminal hydrophilic (KKGRGDS) and N-terminal hydrophobic (V_6) sequences, spontaneously assembled in micelles of 30 nm size in neutral condition, but not at pH 5, whereby the lysine moieties were protonated. The micelles were loaded with DOX. Release of drug was markedly faster at pH 5 than at pH 7. *In vitro* experiments revealed DOX internalisation in $\alpha_v\beta_3/\alpha_v\beta_5$-expressing HeLa cells, but not in integrin-devoid COS7 cells. *In vivo* studies would be helpful in assessing the potential value of this simple peptide.

The acid-sensitive bis-amido *cis*-aconityl linkage of DOX (see mechanism in Fig. 6C) with RGD-labelled PEGylated polyamidoamine (PAMAM) dendrimer was reported by Zhang *et al.* [94], following an early report of *cis*-aconityl-DOX and lysozyme coupling for increasing urinary excretion and concentration at sites of bladder cancer [214]. The new system was investigated *in vivo* for biodistribution and antitumour activity in an orthotopic murine model of C6 glioma. It showed significantly increased half-life and increased AUC of plasma concentration in comparison with free DOX, with accumulation in tumour and prolonged survival. Similarly, *cis*-aconityl-DOX has been combined with iRGD for improving glioma penetration (see Section 8) [93].

Ferromagnetic particles were labelled with both c(RGDyK) and DOX via a poly-L-glutamic cross linker [215]. The particles were acid sensitive and showed enhanced uptake and cytotoxicity in cancer cells expressing integrin $\alpha_v\beta_3$, with respect to non-targeted controls.

A multifunctional NP was fabricated introducing the RGD sequence on a block-copolymer consisting of hydrophilic zwitterionic polycarboxybetaine methacrylate (for prolonged systemic circulation), and of hydrophobic (2-(diisopropylamino)ethyl methacrylate). The latter becomes hydrophilic by protonation and triggers disassembling of NPs and drug release. DOX was loaded at the assembly step. After tests *in vitro* in HepG2 cells, *in vivo* experiments in hepatocellular HepG2 carcinoma-bearing mice confirmed significantly enhanced tumour accumulation at 2 h with respect to free DOX, and at 5 h also with respect to NPs not labelled with RGD [216].

6.2. Drug release by proteinases

Effectiveness of delivery systems may be improved also by responsiveness to enzymatic mechanisms, suitable to cleave off moieties exploited for biodistribution and to expose molecular features relevant to cellular binding and internalisation. Applicable criteria are similar to those of prodrugs (see Section 3). In cancer, attention can be focussed on local expression of proteolytic enzymes, specifically matrix metalloproteinases (MMPs), and cathepsin B.

IONPs have been coated with PEG hydrogels, by photo-initiated polymerisation of acrylate-PEG (acr-PEG), mixed with acr-PEG-RGDS for targeting, and with acr-PEG-GGGPQGIWGQGK-PEG-acr. The latter is cleavable by MMPs at the glycine–isoleucine junction [217]. These particles were suitable for loading DOX (approximately 10%), and inducing DOX uptake into HeLa cells. In comparison with free DOX, a 2.2-fold enhanced uptake was observed, along with 5-fold increase of cellular iron content, whereas no significant uptake of DOX and iron was shown in non-labelled NPs. The differential contributions of RGD targeting and enzymatic cleavage were not fully clarified. *In vivo* experiments are not yet reported.

A sophisticated chemical approach to NPs with a label for targeting $\alpha_v\beta_3$ integrin shielded by an MMP2-cleavable PEG coating is reported by Gianella *et al.* [218]. The core, consisting of nanoemulsion of soybean oil, was coated with a mixture of PEG_{350} phospholipids, cRGDfK-labelled PEG_{1000} phospholipids, and of MMP2-cleavable methoxy-PEG_{2000}-peptide, linked by click chemistry to polyoxyethylene-DSPE (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine) (Fig. 14A). In parallel, particles carrying biotin, instead of the integrin ligand, were prepared for experiments of adhesion on avidin monolayers, proving enhanced adhesion after MMP2 incubation. Loading experiments on RGD-labelled particles were performed with oleic acid-coated IONPs, with oleylmercaptan-conjugated gold NPs, or even with symvastatin, exploring their potential as delivery, diagnostic, or theranostic tools. Targeting experiments by flow cytometry in HUVEC cells, breast cancer MDA-MB231 cells, and J774A1 murine macrophages showed enhanced cell association after exposure to MMP2.

Protease-activatable cell penetrating peptides (ACCP) are constituted by a polycationic cell-penetrating peptide (CPP), typically 6–12 Arg residues, attached to a polyanionic sequence, typically 8 Glu residues, via a protease-cleavable linker, e.g. PLGLAG, cleaved at the Gly-Leu junction by MMP-2 and -9. Thus, enzymatic cleavage could assist the internalisation of a therapeutic agent linked to the cationic sequence [219]. Two studies addressed integrin targeting by decorating the anionic sequence with cRGD [220,221]. They assumed that co-localisation on cells of the hemopexin domain of MMP with integrin $\alpha_v\beta_3$ could favour the targeting of the site of cleavage and then the attachment and internalisation of the cationic sequence and its load consisting in either the NIR probe Cy5, or the anti-tubulin agent monomethyl-auristatin E [222]. Fluorescence results in U87MG glioblastoma cells were confirmed *in vivo* in orthotopic MDAMB-231 mammary tumours. In the same *in vivo* model, methyl-auristatin-linked micelles showed significant tumour regression and increased survival, with complete regression in 28% of treated animals. The results were markedly different from those of the free drug, and of a construct carrying a RAD sequence in lieu of RGD [220]. The value of the approach was confirmed on xenografts of human pancreatic PANC-1 or colorectal HCT-116 tumour, where the potential of radiosensitisation was assessed by comparing the free drug with the loaded micelles, whereby the latter induced a robust and significantly prolonged tumour regression [221].

Coupled targeting of tumour expressions of integrin $\alpha_v\beta_3$ and MMP, was exploited to improve cancer imaging with respect to normal tissue [223,224]. An MMP responsive fluorogenic probe with RGD labelling (Fig. 14B) efficiently targeted *in vivo* $\alpha_v\beta_3$- and MMP-overexpressing U87MG tumours in mice. NIR fluorescence was enhanced and prolonged following the MMP cleavage of the quencher moiety [223]. A multipurpose hybrid peptide c(RGDfE)K(DOTA)PLGVRY has been prepared with c(RGDfE) cyclic peptide for $\alpha_v\beta_3$ targeting, DOTA chelating ^{64}Cu for PET imaging, along with an MMP sensitive sequence that also carried tyrosine, suitably for ^{123}I iodination [224].

DOX conjugated with a GFLG linker, and iRGD conjugated with a GG-PLGLAG linker, were both inserted in a hydroxypropyl-methacrylamide copolymer (Fig. 14C) [97]. The assumption that delivery could be controlled by MMP2 cleavage of PLGLAG in tumour microenvironment, reinforcing integrin targeting and cell penetrating properties of iRGD, and then by intracellular cleavage of GFLG by lysosomal cathepsin B, releasing DOX, was supported by *ad hoc* enzymatic assays. Testing of the system *in vitro*, in DU 145 prostate cancer cells and cellular spheroid, showed enhanced cytotoxicity, and enhanced penetration in spheroids by DOX fluorescence, thus warranting progress in investigation and potentially in extension to other anticancer agents.

6.3. Drug release by cleavage of a disulphide bridge

The inclusion of a disulphide bridge in a delivery system allows modifications by reduction or by trans-thiolation. The latter modality is used for cleaving off a protective PEG cover, linked by an S-S bridge, by exogenous administration of a dose of L-cysteine. Use of cleavable PEG, ensuring persistence in circulation times, and allowing, after cleavage, binding and internalisation at target sites had been previously implemented via pH or enzyme sensitive links (see Section 5) [192].

6.4. Combined thermal and pH responsive cleavage

Quan *et al.* introduced the concept of dual sensitivity to endogenous stimuli, involving thermoinduced release of a load of doxorubicin consequent to destabilisation of micelles formed by a non-covalently bound pair of polymers [225].

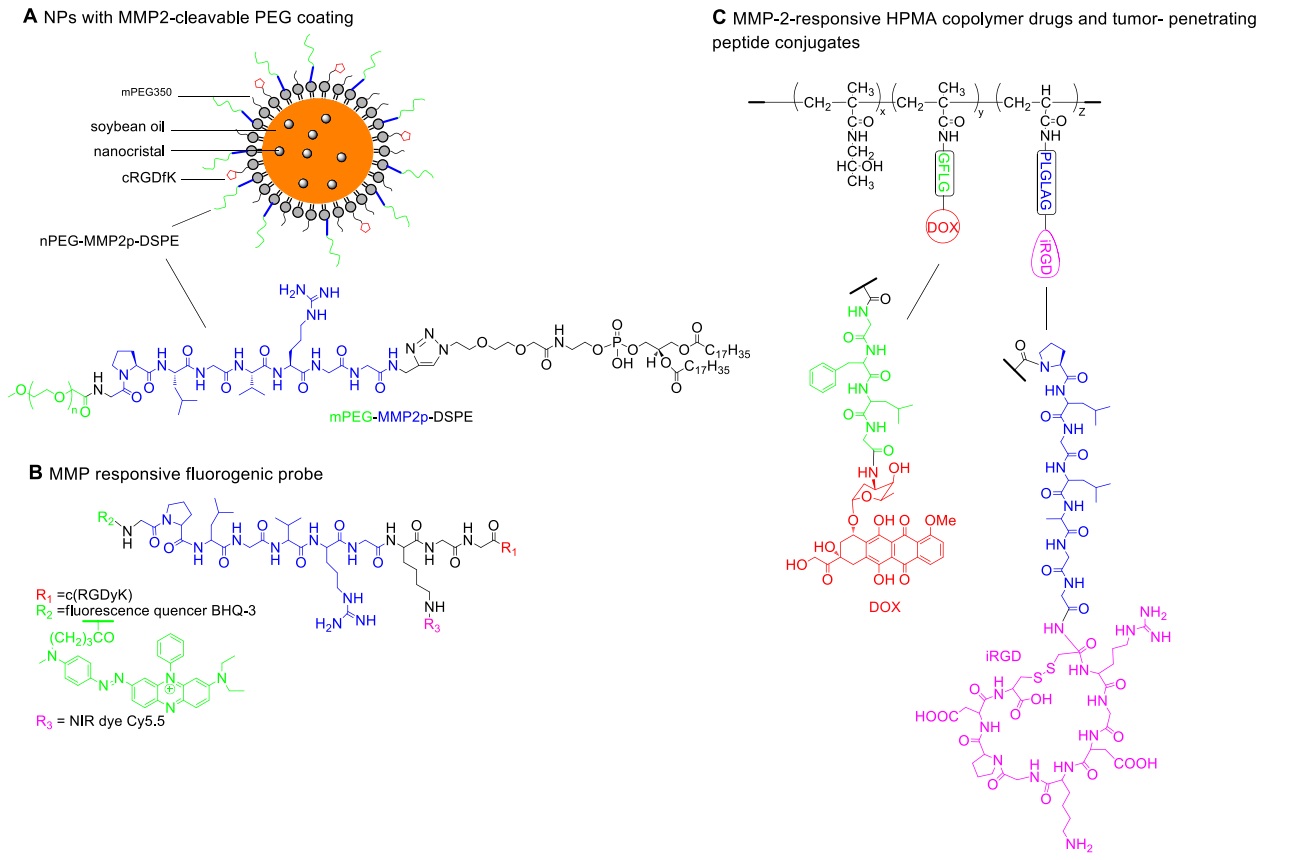

Fig. 14. Integrin targeted delivery systems with proteinase cleavable linkers. A) Soybean oil nanoemulsion coated with a mixture of PEG_{350} phospholipids, and with cRGDfK-functionalised PEG_{1000} phospholipids, as well as MMP2-cleavable methoxy-PEG_{2000}-peptide [218]. B) $\alpha_v\beta_3$ targeted, MMP responsive fluorogenic probe [223]. C) HPMA copolymer conjugated to DOX and iRGD, respectively by chatepsin B and MMP cleavable linkers [97].

Lipophilic beta-cyclodextrin and hydrophilic alpha-cyclodextrin, connected by click-chemistry linkage, were respectively able to accommodate lipophilic polycaprolactone with adamantyl terminal and hydrophilic N-isopropylacrylamide-co-N-acryloxysuccinimide with phenyl terminal. The hydrophilic section was also linked by an imine bond with 4-formylbenzoyl-$MPEG_{2000}$ and with the integrin-targeting peptide NH_2-GRGDS-COOH (Fig. 15). The construct spontaneously assembled in micelles with a hydrophobic core, suitable for DOX loading. It was shown that, in tumour acidic environment, the loss of PEG shield exposed the targeting label, and shifted the critical temperature of the micelles from 38 to 35.5 °C. At increased thermal sensitivity, the construct collapsed with consequent DOX release. Physical characterisation for pH and thermal sensitivity was followed by assays of internalisation in HeLa cells via DOX fluorescence [225].

Thermal and acidity sensitiveness of a nanogel of N-isopropylacrylamide-co-acrylic acid were implemented with iRGD for targeting and internalisation of DOX and a gold probe [184]. DOX was loaded via ionic interaction on the swollen nanogel at pH 7.4. Luminescent gold nanoclusters encapsulated in BSA were conjugated with the gel and decorated with iRGD. pH-dependency of DOX release at 7.4 and 5.2 was assessed in solution at 37 °C, whereas the particles were shown to collapse above 37 °C. Cytotoxicity on HUVEC and mouse melanoma B16 cells, along with uptake and localisation in lysosomes in these cells were proven *in vitro*, but *in vivo* results are not yet reported. A similar approach was suggested for gold nanoclusters encapsulated in ferritin nanocages [226].

7. Physically detectable or modifiable delivery systems

Studies on drug release from delivery systems triggered by different physical stimuli (heat, ultrasound, magnetic field, visible light, ultraviolet or near infrared radiation) have been reviewed [210,227]. Nevertheless, application in integrin-targeted systems is still scarcely explored, whereas diagnostic tools combining integrin targeting with imaging probes for MRI, radioactive isotopes for SPECT and PET, NIR, and ultrasound techniques have been actively pursued in oncological and cardiovascular fields. This work, already extensively reviewed [7,14,228,229], is not discussed here. At present, the potential of exploiting the available techniques for therapeutic purposes is prompting work on targeted delivery involving physical methods for therapy, or diagnosis, or both. In the following, we discuss the matter, including the "theranostic" approach. The latter is inspired by the concept that combining diagnostic and therapeutic tools in targeted delivery systems may afford a perfect bullet with dual value. This indeed could be the case with a theranostic tool for once-only intervention, e.g. in localising and dissolving a thrombus in vascular occlusion by ultrasound irradiation of a microbubble targeting integrin $\alpha_{IIb}\beta_3$ [228,230,231] (see Section 7.4). In most real situations, diagnosis and therapy need to be enacted in separate moments, e.g. when diagnostic controls are applied before and after a cycle of therapy. On the other hand, at least in an experimental set, repeated diagnostic controls may assess the time course of response to a single drug administration. In a study by Bai *et al.* [232], the enhancement of angiogenesis by a treatment with endothelial progenitor cells (EPC) was checked in a stroke model in mice, using Gd-MRI and rhodamine-NIR nanoprobes on (G5) PAMAM dendrimer, targeted to $\alpha_v\beta_3$ integrin by cRGDyK. Integrin expression on neovessels was assessed at 3, 7, 10, 14, and 21 days after induction of stroke followed by single EPC infusion.

However, a clinically validated, targeted diagnostic probe, preserving the same distribution and uptake features, could provide essential information for the design of a therapeutic system even when administered repeatedly. Then, therapeutic results could be confirmed with the diagnostic tool. We would describe such an approach as "two stages

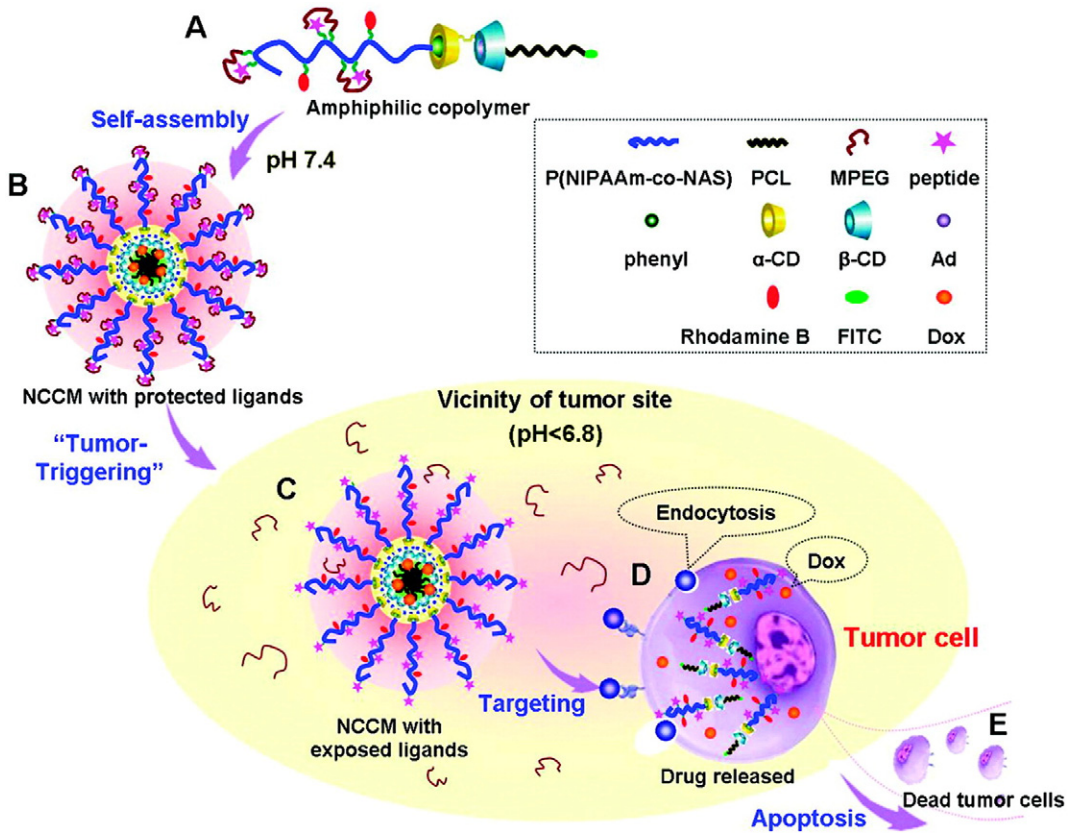

Fig. 15. A) Diblock copolymer formed by non covalent bound with cyclodextrin dimer and employed in preparation of integrin targeted micelles. (Ad = adamantly, P(NIPAAm-co-NAS) = N-isopropylacrylamide-co-N-acroyloxysuccinimide polymer). B) Micelle destabilisation and drug release mechanism. Reprinted with permission from ref. [225]. Copyright (2010) American Chemical Society.

theranostics". Peiris *et al.* [233,234] provide an example in the studies. An integrin $\alpha_v\beta_3$-targeted iron nanoparticle, built by linearly connecting four iron oxide nanospheres in "nanochains" was designed for specifically targeting micrometastases in early phase, in the assumption that shape and multiple RGD labelling could jointly reinforce imaging and drug delivery. [233,234]. Elongated nanoparticles, with respect to spherical particles, enhance vascular interactions and sensing of endothelial cells by moving through microvessels in lateral drift [235]. Iron particles were sequentially connected by solid phase chemistry [236] and then decorated with c(RGDfC) on PEG-NH_2 appendages. At first the particles were labelled with an NIR probe for non-invasive imaging experiments by fluorescence and MRI [233]. In a second study [234], nanochains were constituted of three iron particles and a DOX-loaded liposome. DOX release was triggered by oscillations induced by radiofrequency on iron particle. Exhaustive experiments were performed *in vitro* and *in vivo* in both studies. In a metastatic model, female mice were orthotopically inoculated with breast cancer 4T1 cells. After 2 weeks, the developed primary tumour was removed and, after some days, i.v. nanochain injections followed by radiofrequency stimulation were administered 4 times at 3-day intervals. Of the treated animals, 57% survived at 150 days, and the non-surviving had increased mean survival time of 68 days, whereas the free DOX-treated animals were all dead at 35 days, with an increase of mean survival of 28.5 days. Remarkably, nanochains carried a dose of DOX of 0.5 mg/kg, whereas free DOX was administered at 5 mg/kg,

7.1. Thermosensitive devices

A nanogel of N-isopropylacrylamide-co-acrylic acid collapsing at moderately increased temperature, either in inflamed tissues or by external heating by ultrasound, has been exploited in a construct combining iRGD, DOX, and luminescent gold nanoclusters (see Section 6.4) [184].

Thermosensitive liposomes, delivering DOX under externally induced hyperthermia (42 °C), were prepared by adding an elastin like polypeptide (stearoyl-NH-VPGVG-$CONH_2$) in the liposome surface. The liposome included 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-PEG_{2000} conjugated with cRGDyK [237]. *In vitro* experiments confirmed integrin targeting, temperature dependent release, and enhanced cytotoxicity in $\alpha_v\beta_3$-expressing U87MG cells. Authors express hope that future studies, using ultrasound or radiofrequency stimulation, would afford progress with respect to a non-targeted system, discontinued in phase III studies for inadequate results in hepatocellular carcinomas.

7.2. Bimodal imaging with pH-inducible drug release

Yang *et al.* constructed a carrier with multiple properties: integrin targeting, high load of DOX with pH sensitive binding, MRI and PET detection, as well as good solubility, *in vivo* stability, and long circulating time [95]. Ferromagnetic NPs with lipophilic cover were bound by catechol–iron chelation to 6-hydroxydopamine, providing amino function for coupling, via NHS activation, of methoxy-PEG, maleimide-PEG, and monomethyl malonate. Malonic ester was linked via hydrazide to DOX. PEG was conjugated via maleimide to cRGDfC and to NOTA-SH, the latter ready for chelation of ^{64}Cu for PET. DOX fluorescence detection in U87MG cells showed that RGD-labelling doubled the uptake. Assays of intracellular distribution showed localisation in cytoplasm, whereas free DOX accumulated in the nucleus. Cytotoxicity was comparable with that of an equivalent dose of free DOX, but afforded a 20-fold increase with respect to non-RGD-particles. ^{64}Cu-microPET in mice with subcutaneous transplants of U87MG glioma indicated specific tumour uptake, suppressed by co-administration of free cRGD. A second study

[96] adopted a similar approach, except for MRI functionalisation, in hyperbranched amphiphilic block-copolymer H40-poly(L-glutamate-hydrazone-DOX)-b-poly(ethylene glycol), conjugated with cRGDfC and NOTA for ^{64}Cu chelation. Again, superior uptake with respect to non-targeted particles was assessed in U87MG tumour-bearing mice.

7.3. Theranostic approaches

Melancon *et al.* reviewed the critical issues for the development of nanotheranostics for cancer [238], and reported on their studies on photoacoustic imaging and photothermal ablation therapy with gold NPs. These optionally included PET probes or magnetic NPs for MRI [239,240]. An important study involved integrin targeting for photothermal ablation by NIR laser irradiation of orthotopic gliomas in mice (see Section 8) [239].

Incorporation of a fumagillin prodrug (see Section 3.5) in copper-phospholipid NPs with $\alpha_v\beta_3$ targeting provided a theranostic tool for photoacoustic imaging of neoangiogenesis and therapy in cancer and atherosclerosis [241].

Integrin-targeted PTX-albumin NPs were obtained by a technologically feasible approach [185], owing to the spontaneous assembly, by simple mixing, of PTX and two modified albumins. These were respectively conjugated with the porphyrin-based photosensitising agent chlorine e6 and with a thiolated derivative of cRGDyK for $\alpha_v\beta_3$ targeting. Chelation of divalent Mn^{2+} in the porphyrin cage before assembly with PTX afforded NPs with additional MRI imaging value. Assays in human glioma U87MG cells supported the premises, including integrin targeting (assessed by fluorescence and MRI imaging) and enhanced cytotoxicity with respect to non-targeted NPs. *In vivo* fluorescence, in nude mice bearing U87MG implants, confirmed a 2.4 higher uptake of targeted particles in tumours. Moderate inhibition of tumour growth was observed only in the early days after administration, whereas photodynamic irradiation at 660 nm induced prolonged remarkable effects, leading to the survival of all treated animals at 40 days, thus proving the theranostic assumption, with the limit of light penetration in tissues.

Aiming at imaging and therapy of bladder cancer, Lin *et al.* developed micelles loaded with PTX or daunorubicin, decorated with the PLZ4 peptide (sequence cQDGRMGFc, where c represents D-cysteine used for cyclisation, 8 Fig. 1), as well as with the fluorescent dye DiD [60,61]. The peptide had shown an affinity for $\alpha_v\beta_3$ integrin in various bladder cancer cells [59]. Following *in vitro* testing in dog bladder cancer cells [60], the PTX-loaded particles were applied *in vivo*, in models of orthotopic implants of human bladder cancer [61] in mice. The implants were established either from the 5637 cell line, or from cells of human cancer specimens. Efficacy was indicated by delay of tumour growth and prolongation of survival. It may be observed that fluorescence accumulation in these cancers indicates a theranostic potential, since it could be used clinically in visual detection in bladder.

7.4. Imaging and delivery with microbubbles

Microbubbles (MB) of perfluoroalkane gas enclosed in phospholipid shells, or albumin shells, distribute in the circulatory system, without extravasation, because of their size, ranging from 1 to 10 μm. MB are suitable for loading drugs, or targeted delivery systems, and can also carry targeting moieties on their surface. MB reflect ultrasound ("contrast enhanced ultrasound"), resulting in excellent echographic imaging, and can be disrupted by external irradiation with ultrasound bursts of increased energy [242], releasing their content. This system offers an attractive theranostic approach, applicable in vascular diseases and in neoangiogenesis of cancer.

Targeting integrin $\alpha_{IIb}\beta_3$ in thrombosis can be favoured by the retention on binding sites in microvessels for imaging or delivery [243]. A notable example of effective single-treatment theranostic was offered by integrin targeting in pigs with coronary occlusion, followed by local disruption and release of loaded prourokinase ("sonothrombolysis"), affording epicardial recanalisation and microvascular recovery [230]. It may be noted that recent advancements about $\alpha_{IIb}\beta_3$ antagonists devoid of the side effects of existing drugs [244] may also open the way to new delivery systems with dual action or theranostic potential.

Early experiments of MB targeting $\alpha_v\beta_3$ integrin [245,246] are actively prosecuted in the light of results with the technique of "sonoporation" by disruption of MB, inducing temporary permeabilisation of membranes, specifically at the blood–brain barrier, that may allow penetration of loaded, or co-administered drugs (see Section 8) [247]. An example is provided by the delivery of (hVEGF)-siRNA from integrin $\alpha_v\beta_3$-targeted NPs in PC3 prostate cancer cells *in vitro*, reinforced by disruption of co-administered MB [248].

8. Drug delivery to brain tumours

Cilengitide, an $\alpha_v\beta_3$-integrin antagonist widely investigated in angiogenetic processes and in various cancers, has been the only integrin antagonist reaching advanced clinical trials in cancer, and specifically in gliomas. It was administered by i.v. infusion in large doses (e.g. 2000 mg twice weekly), with good tolerability and some encouraging results in a number of studies, alone or in combination with radiotherapy or temozolomide, and finally in phase III trials [249], but its development has been abandoned [7].

Drug treatment of brain cancers, particularly of gliomas, and of metastases from primary tumours of other organs (most frequently from lung, and from melanomas or breast cancer [250]), represents a main challenge in therapy, because of the difficulties of delivering drugs across the blood–brain-barrier (BBB) membrane via systemic administration [251]. Research, in recent years, addressed the intertwined problems of targeting tumours and overcoming BBB. Studies were encouraged by the results obtained with cilengitide, thus focussing on targeting integrin $\alpha_v\beta_3$ either with prodrugs or with particulate delivery systems. Examples with interesting results, although in a preliminary stage, were discussed in previous sections for PTX [24,25], PTX-albumin particles [185], Pt(IV) prodrugs [116,117], DOX conjugated with tissue-cleavable bonds [93–96], ^{64}Cu-PET imaging on graphene oxide or on ferritin particles [143,169].

8.1. Labelling for integrin and transporter receptors

The transport of RGD-PTX prodrugs was attempted by targeting transporter receptors for folic acid [252] or transferrin [253] in the BBB. A prodrug coupling PTX-2′-succinate and cRGDfK was encapsulated in hybrid polymer–lipid particles of phosphatidylethanolamine-PEG_{2000}, decorated with folic acid. These particles afforded marked prolongation of survival of mice with orthotopic implants of human glioblastoma T98G, with respect to the prodrug, and significantly improvement with respect to particles lacking folic acid [252].

Hybrid micelles loaded with the prodrug were also conjugated with transferrin, and were evaluated in mice bearing intracranial U87MG glioblastoma. They were significantly more effective in prolonging survival than free PTX or particles loaded with PTX, either with or without transferrin coupling, thus pointing the contribution of integrin targeting. Retention in tumours was also improved with respect to all control groups, whereas toxicity was decreased, as indicated by the preservation of body weight [253].

A different approach appended cRGDfK integrin ligand on the surface of micelles of PEG-polyglutamate copolymer incorporating (1,2-Diaminocyclohexane)platinum(II) [254]. Efficacy and accumulation in tumour were investigated in an orthotopic model of human glioblastoma U87MG, in comparison with particles carrying RAD sequence instead of RGD. cRGDfK fuctionalised particles were markedly better than the RAD labelled ones, both in accumulation and in reducing tumour volume, confirming the relevance of integrin targeting in this

setting. No inhibition of tumour growth was observed in oxaliplatinum-treated animals.

8.2. Antibodies of transferrin receptor for oligonucleotide transport

Antibodies of transport receptors can assist delivery systems in overcoming BBB and blood-tumour barriers (BTB). An approach by multiple mechanisms for carrying two antisense oligonucleotides (AONs) across BBB and inside glioma cells [255] used prior experience of labelling with antibodies [256]. The target was not an integrin, but integrin-related laminin-411, an ECM protein overexpressed in angiogenesis, and essential in tumour development. The aim was inhibition, by the AONS, of the synthesis of the α_4 and β_1 subunits of the protein. The multiple purpose required NHS-conjugation of the carboxylic groups of poly-beta-L-malic acid with NH_2-Leu-Leu-Leu-OH, NH_2-Leu-Leu-Leu-NH_2, NH_2-Leu-ethylester, and 2-mercapto-1-ethylamine. The sulfhydryl group of the latter was used for linking, via disulphide bridges, thiolated morpholino AONs, antibodies, and optionally the fluorophore Alexa Fluor 680 (Fig. 16).

A transferrin antibody and the anti-nucleosome antibody 2C5 were linked on the polymer. The system was also equipped with lysogenic leucine-carrying peptides, activatable by local acidity for escape from the endosomal system into the cytoplasm, where AONs had to be released by glutathione splitting of S-S bridges. AON inhibition of laminin-411 synthesis was assessed *in vitro* in human U87MG and T98G glioma cells, and cellular uptake, endosomal localisation and escape to cytoplasm were assessed by *ad hoc in vitro* experiments. *In vivo* efficacy was assessed by multiple treatments (8 times at 3 day intervals) in mice bearing intracranial human glioma U87MG, resulting in 90% smaller tumours than in control animals. Controls with delivery systems devoid in turn of AONs, or antibodies, or leucine-carrying sequences proved the active role of these components in the overall effect. The results were taken as a proof of therapeutic relevance of inhibition of laminin-411, and as an evidence supporting the construction of delivery systems with appropriate drugs for therapeutic progress in other CNS pathologies, importantly in multiple sclerosis and Alzheimer disease.

8.3. Targeted irradiation therapy

The experience of physically-induced release of DOX by external radiofrequency of integrin $\alpha_v\beta_3$-targeted iron nanochains-DOX-liposomes [234] (Section 7) was extended to orthotopic glioma models, affording marked increase in local drug concentrations [257].

Photothermal ablation by NIR laser irradiation of hollow gold nanospheres in glioma models is reported [239]. The nanospheres can be used for generating intense photoacoustic signals, and for photothermal ablation therapies. Integrin targeting was achieved with c(RGDfK) conjugated with N-hydroxysuccinimidyl-PEG-S-acetylthioacetate, followed by attachment on gold of free thiol group, deprotected with hydroxylamine. Orthotopical gliomas in mice were induced by inoculation of human glioblastoma cells. The cells were transfected with luciferase gene (U87-TGL), in order to assay tumour growth by bioluminescence under D-luciferin treatment. Contrast-enhanced photoacoustic images were taken 24 h after i.v. injection of the particles. In parallel, particles were also labelled with ^{64}Cu and injected for microPET imaging. Mice were submitted to NIR laser irradiation for tumour ablation, and then followed for survival. MicroPET and photoacoustic imaging consistently showed localisation in tumours. Thermal ablation approximately doubled the survival time of treated animals. The bioluminescence assays showed regression of tumours for 10 days, but thereafter a trend to increase, indicating recurrence.

8.4. Proapoptosis with iRGD by "bystander" effect

Investigations in glioma models of novel peptides with peculiar properties, in combination with the cell-penetrating iRDG peptide, are described in detail [199]. Elongated IONPs, dubbed "nanoworms", with 80–100 nm length and 30 nm thickness, were multiply decorated with PEG_{5000} linked with the chimeric conjugate CGKRK-$_D$(KLAKLAK)$_2$ (Fig. 17). This peptide combines features of CGKRK and $_D$(KLAKLAK)$_2$. The former has homing properties in tumour endothelia and cells, whereas the latter can disrupt bacterial membranes, as well as eukaryotic mitochondrial membranes. The mitochondrial protein p32/gC1qR/HABP, also expressed on the surface of endothelial and tumour cells, has been identified as the target of CGKRK [258].

In vitro experiments in HUVEC and T3 glioblastoma cells with particles carrying the chimeric peptide showed 100-fold apoptotic potency in comparison with the free peptide. Effects persisted after washing of the cells, at difference from particles decorated with only one peptide.

In vivo experiments were performed in two models of intracranially implanted murine glioblastomas, resembling human tumours in aggressiveness and brain spreading. In the first model nanoparticle with chimeric peptide afforded almost full protection and survival, whereas

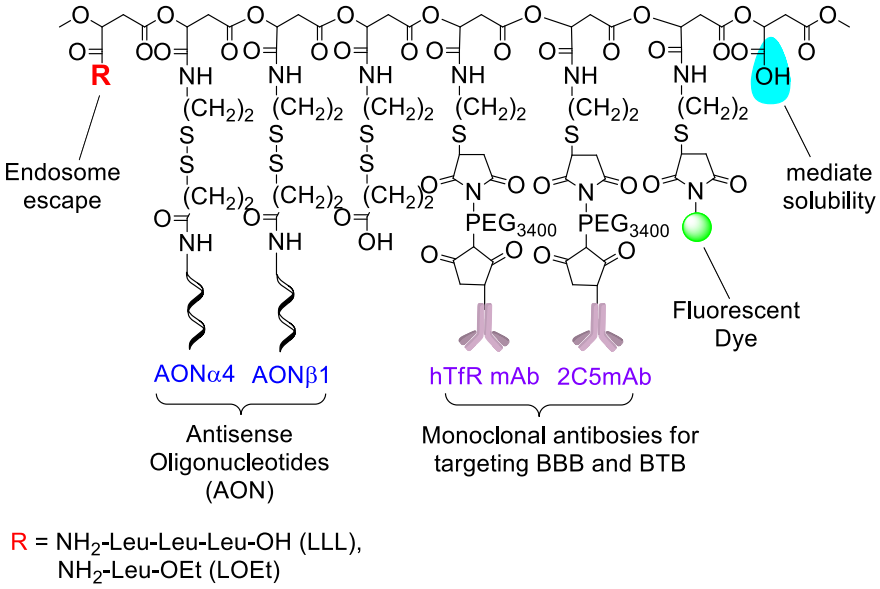

Fig. 16. Polymeric multifunctional delivery system carrying AONs for inhibition of synthesis of laminin-411, mAbs for targeting BBB and BTB, and the NIR dye Alexa Fluor 680 [255].

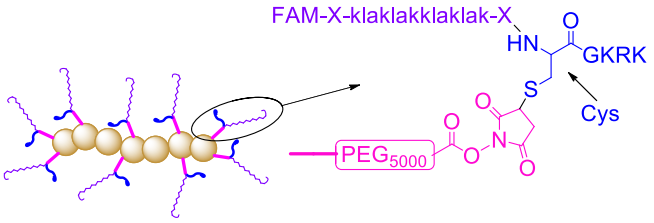

Fig. 17. Chains of IONPs ("nanoworms") conjugated via a PEG_{5000}-NHS linker with the peptide CGKRK-$_D$(KLAKLAK)$_2$ combining the homing peptide CGKRK and the proapoptotic peptide $_D$(KLAKLAK)$_2$ [198,199].

particles with $_D$(KLAKLAK)$_2$ did not differ from controls. In the second model, the median survival of the animals was extended to 52 days, with respect to approximately 30 days of both control and $_D$(KLAKLAK)$_2$ particles. Interestingly, the combined treatment with iRGD further enhanced median survival to 85 days, whereas no enhancement was observed in the combined treatment with CRGDC. Agemy *et al.* [198] had reported similar improved efficacy, due to the "bystander effect" of iRGD co-administration in glioblastoma. It appears probable that the enhanced efficacy might depend upon improved internalisation in tumour cells, and not just in vessels. Authors concluded that these results encourage developments towards clinical testing in glioblastoma, and prompt investigation of the "bystander effect" in other cancers as well.

8.5. $\alpha_2\beta_1$ integrin targeting

In addition to integrin $\alpha_v\beta_3$, other integrins expressed in gliomas deserve attention for drug targeting [259]. Observations of β_1 integrin upregulation in bevacizumab-resistant glioblastoma found confirmation of clinical relevance when intracranial administration of an anti-β_1 antibody in mice reduced tumour growth and reinstated bevacizumab responsiveness [260].

A theranostic approach to $\alpha_2\beta_1$ expressing tumours, combining ^{68}Ga PET imaging and microbubble disruption is reported [86]. Microbubble sonication was performed either on standard microbubbles co-administered with the integrin-targeted imaging tool, or by embedding the tool in microbubble membranes. In both cases significant enhancement in tumour uptake was observed. Progress with drug loading for proving efficacy could open interesting perspectives for this approach.

9. Integrin targeting of gene-mediated therapies

Gene therapy is based on the delivery of functional genes into cells for substitution or integration of defective or missing genes, in order to restore the functions controlled by the proteins encoded by such genes. Clinical applications were first envisaged for the treatment of hereditary conditions, such as haemophilia, but deeper understanding of the genetic bases of other pathologies expanded research on gene therapy to a wider spectrum of diseases, including cancer.

The main limitations in therapeutic applications reside in difficulties of transporting complex DNA sequences in intact form to cellular nuclei, overcoming extracellular and intracellular barriers. Efforts have been focussed on the development of delivery system appropriately designed for gene delivery to target cells without degradation, avoiding the problematic use of viral vectors. [261]. In this direction, integrin targeting of gene delivery in cancer therapy received attention since 2002, with the first experimental example of targeting integrin $\alpha_v\beta_3$ on tumour neovascularisation [262]. Hood *et al.* showed pronounced tumour regressions in mice by systemic delivery of RGD-labelled cationic NPs loaded with the mutant Raf-1 gene, modulating the cascade of two key factors of angiogenesis, basic fibroblast growth factor (bFGF) and vascular endothelial cell growth factor (VEGF). A number of investigations of integrin-targeted gene delivery systems followed this first example, but they were tested only *in vitro* in cellular models [7,16,263].

In recent years, however, the expectation of advancement in gene-mediated therapies shifted its focus on new approaches of modulation of gene functioning by epigenetic modifications with RNA oligonucleotides (ONs) with relatively short sequences with respect to full genes, namely antisense oligonucleotides (AONs) and gene silencing siRNAs. Both ON types do not need to enter the cell nucleus, because they act by down-regulating expression and functions of the proteins encoded by the relevant genes [264]. Their mechanisms of action, and their synthesis with appropriate structural modifications, designed for improving stability with respect to the natural sequences, have been reviewed in detail [264,265], along with relevant biological experiments. Main problems, such as the intrinsic limitations of cell contact and internalisation, due to their negative charge, and risks of adverse effects, because of scarce specificity and unwanted immunological responses, were pointed out, along with efforts of overcoming these difficulties with appropriately designed delivery systems, mostly based on NP carriers.

Progress in this direction appears especially warranted in the therapy of cancer, in the hope of finding effective drug with good tolerability, as an alternative to the use of potent cytotoxic agents of the available armamentarium. We intend now to focus on recent developments of targeted delivery of ONs aimed at assessing the value of gene modulation in cancer therapy by targeting the expression of $\alpha_v\beta_3$ and related integrins in tumour cells and stroma. These studies, in particular when showing *in vivo* efficacy in tumour models, but also when limited to model experiments of fluorescence and to cancer cells *in vitro*, appear as promising steps towards future progress and clinical applications.

9.1. siRNA delivery

A straightforward, "prodrug-like", approach to integrin targeting of siRNAs employed the direct conjugation of the properly functionalised ON with an integrin ligand. In a proof-of-concept model, Juliano and co-workers adopted the strategy of combining multivalent cRGD, using 2–4 copies of the ligand, with luciferase siRNA (Fig. 18A). The construct, with fluorescence labelling, was tested on $\alpha_v\beta_3$-expressing M21$^+$ human melanoma cells [266], affording evidence of enhanced uptake with respect to non-conjugated siRNA. Minor differences in internalisation rates were observed for bi-, tri-, and tetravalent cRGD conjugates. Still, a dose-dependent reduction in luciferase expression was observed with the tri- and tetravalent conjugates (up to 70% for the trivalent conjugate at 25 nM), whereas the bivalent version had little effect.

In a similar way, a modified thiolated VEGFR-2 siRNA was directly conjugated with maleimido-functionalised integrin ligand cRGDfK (Fig. 18B) [267]. 2′-O-Me-ribose modifications of three nucleotides on each end of both strands were used for enhancing resistance to RNA nucleases and minimising any non-specific activation of toll-like receptor-mediated immune response. Silencing of VEGFR-2 was expected to inhibit angiogenesis and consequent tumour growth. Indeed, the construct was able to silence VEGFR-2 expression in HUVECs with maximum knockdown of mRNA and protein occurring respectively at 48 and 72 h of exposure and persisting up to 120 h. It induced irregular angiogenesis in zebrafish embryos 72 h after treatment, differently from the controls treated with non-targeted siRNA. *In vivo* biodistribution and gene knockdown of two different chemically stabilised VEGFR-2 siRNAs, both with cRGDfK conjugation, were assessed by whole animal bioluminescence in nude mice bearing human luciferase-expressing A549 tumours. Both conjugates downregulated the corresponding mRNA (55% and 45%) and protein (65% and 45%) in tumours, and markedly reduced tumour volume with respect to control groups (cRGD, cRAD-siRNAs, cRGD-control siRNAs). Moreover, no innate immune response was observed.

Other studies approached the delivery of siRNAs using integrin-targeted NPs, either by chemical conjugation, or by loading into the particles. A favourable opportunity is provided by the complexation with

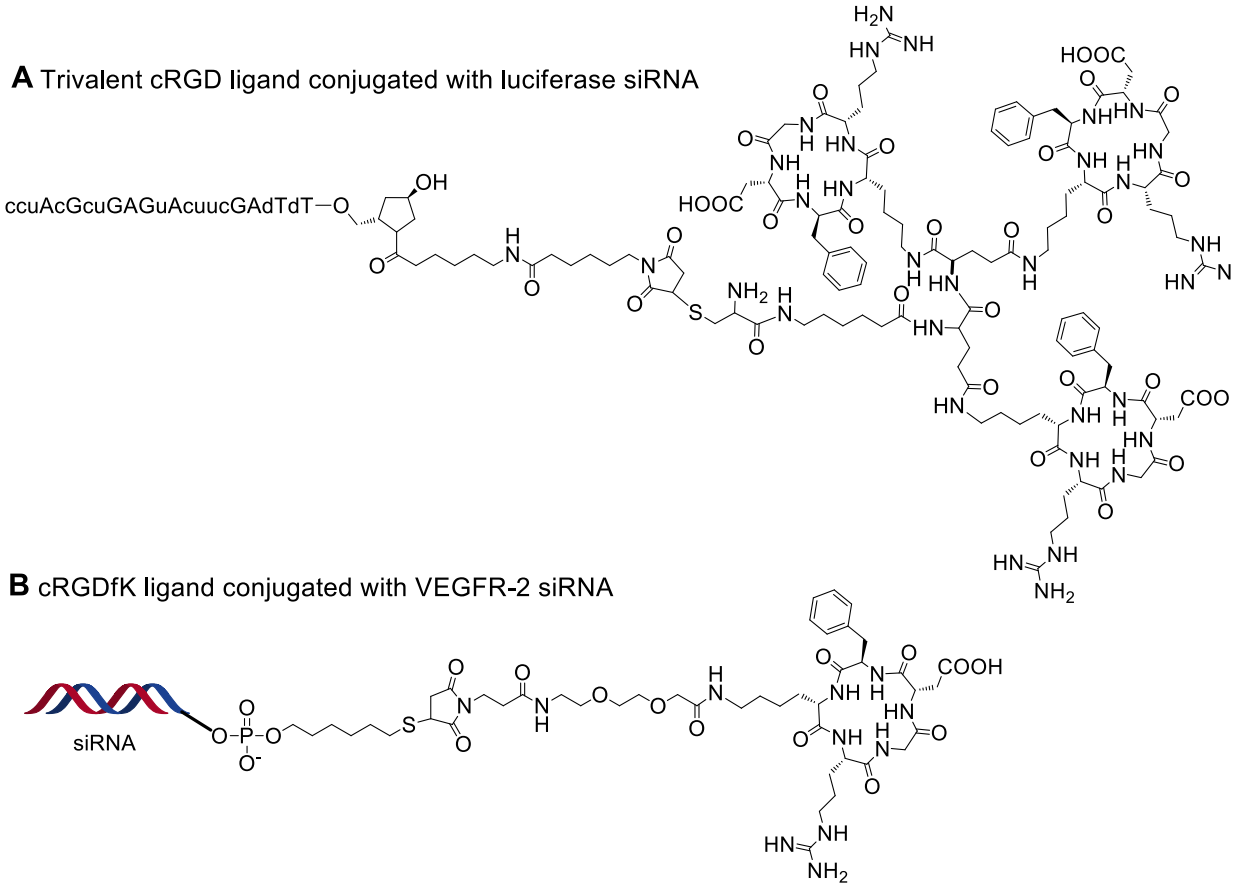

Fig. 18. siRNA delivery. A) Trivalent cRGD ligand conjugated luciferase siRNA [266]. B) VEGFR-2 siRNA conjugated with maleimido-functionalised integrin ligand cRGDfK [267].

charged polymers, e.g. polyamidoamine (PAMAM) dendrimers, which are among the most employed NP platforms for ON delivery [268]. These highly cationic polymers enable complexation of ON by electrostatic interactions, and are endowed with surface functions suitable for conjugation of targeting ligands.

Li *et al.* linked a G4 PAMAM dendrimer with a cyclic RGD ligand using an NHS/maleimido bifunctional PEG. [269]. Dendrimer, carrying 19 PEGs and 12 cRGD moieties, was complexed with siRNA for the K^+ channel-encoding ether-à-go-go-related gene (*hERG*), playing a role in cancer cell proliferation, invasion, and metastasis [270]. Cellular internalisation and gene silencing of the complex were assessed by flow cytometry and polymerase chain reaction in human anaplastic thyroid carcinoma (ATC) HTC/3 cells, showing the downregulated expression of *hERG* to 26.3% of the control values, with consequent apoptosis, thus warranting further investigation.

A complex of triethanolamine-core G5 PAMAM dendrimers with Dicer-substrate siRNA (dsiRNA), suitable for silencing the heat shock protein 27 (Hsp27) gene, was labelled with the dual peptide $E_{16}G_6$RGDK. The latter include the iRGD peptide for targeting $\alpha_v\beta_3$ and cell penetration (Section 5) [104], along with negatively charged 16-(amino acid) polyglutamic acid (E_{16}), apt to binding of positively charged siRNA/dendrimer, and a 6-unit polyglycine (G_6) linker [271]. The construct displayed 2-fold enhanced cellular uptake in comparison with the non-labelled complex, resulting in more effective gene silencing in prostate cancer PC-3 cells *in vitro*. *In vivo* in nude mice bearing PC-3 xenografts almost 2-fold gene silencing enhancement was observed with the targeted construct, with improved inhibition of cell proliferation and tumour growth.

The other well-known cell penetrating peptide TAT was employed in an RGD4C-labelled siRNA carrier aimed at overcoming P-glycoprotein mediated resistance (Section 5.1) [37].

Improved transfection efficiency of a construct of a VEGF plasmid siRNA loaded on bio-reducible poly(cystamine bisacrylamide-diaminohexane) polymer [272] was sought by conjugation with a PAMAM G0 dendrimer and cRGDfC-labelling [273]. The final construct showed 20%–59% higher uptake into $\alpha_v\beta_{3/5}$-expressing breast cancer MCF7 and pancreatic cancer PANC-1 cells with respect to the non-targeted construct. Comparative experiments with $\alpha_v\beta_{3/5}$ integrin-negative 293 cells resulted in significantly lowered VEGF gene expression (51%–71%) and cell viability (35%–43%) in the integrin expressing cells.

A highly branched histidine–lysine-rich peptide is an effective siRNA carrier with low toxicity [274]. Lysine residues presumably bind and protect siRNA via electrostatic interactions, meanwhile pH-sensitive histidines buffer endosomal acidity, helping endosomal escape of siRNA. However, its PEGylation appeared to hinder cell targeting. Therefore, in a second study, four cyclic RGD sequences were conjugated at the terminals of the PEG chains [275]. The new construct was loaded with siRNA for luciferase gene quenching. It inhibited luciferase expression in mice xenografted with MDA-MB-435 tumours, having 4-fold higher blood levels with respect to a non-targeted construct, with 40% higher accumulation in tumour tissue, and 60% lower luciferase activity. When this construct was loaded with siRNA for the Raf-1 oncogene, tumour growth, in mice carrying MDA-MB-435 tumours, decreased by 35% with respect to the non-targeted construct and by 60% with respect to non-treated animals [276].

Natural cationic polymers have been extensively employed for ON delivery [277]. Han *et al.* targeted chitosan NPs by cross-linking of amino groups of cRGDfK integrin ligand with those of chitosan, and loaded them with three different siRNAs [165]. Targeting was ascertained by testing in $\alpha_v\beta_3$-positive SKOV3ip1 vs. A2780ip2 $\alpha_v\beta_3$-negative cells. Efficacy of the construct in silencing different

growth-promoting genes (POSTN, FAK, and PLXDC1), all relevant in ovarian cancers, was assessed *in vivo* in orthotopic models of the tumours. Integrin-targeted POSTN-siRNA particles were markedly effective in mice bearing POSTN and $\alpha_v\beta_3$-positive SKOV3ip1 tumours, reducing POSTN expression by over 51% and tumour growth by 71%, with respect to unlabelled NPs, whereas either targeted and non targeted particles produced similar effects in mice bearing $\alpha_v\beta_3$-negative A2780ip2 tumours. A similar picture emerged with FAK-siRNA loaded, integrin targeted particles in mice bearing FAK and $\alpha_v\beta_3$-positive HeyA8 tumour. Finally, enhanced inhibition of tumour growth was observed by delivering PLXDC1-siRNA RGD-labelled NPs in mice bearing $\alpha_v\beta_3$-negative A2780 tumours, when compared with PLXDC1-siRNA delivered with non-targeted particles, suggesting ability of these NPs to target integrins expressed in tumour neovessels even without specific expression in tumour cells [165].

Among NPs used in ON delivery, inorganic particles increasingly attract attention of investigators, as exemplified for gold, for MRI detectable manganese–iron oxide particles, or for fluorescence models with quantum dots.

Gold NPs are employed in gene or ON delivery, because of easiness of chemical conjugation on their surface of both nucleotides and targeting moieties [278,279]. De la Fuente and co-workers employed integrin-targeted gold NPs in the delivery of c-myc siRNA [280]. RGD sequences were introduced at the terminals of short PEG chains layered on the surface. In a preliminary study, c-myc siRNA was either loaded by ionic interaction with quaternary ammonium functionalised NPs or covalently attached to gold surface through a 5′-thiol function in the ON. Whereas the first NP type was found effective in gene silencing only in cells and in simple *in vivo* invertebrate model (freshwater polyp, Hydra), the covalently bound type was able to ensure active siRNA release in mice, with 65% c-myc silencing, vs. 35% of the first type, as assessed in excised lung specimens following intratracheal administration. The second was further evaluated *in vitro* in three different cell lines (CCL-206 lung fibroblasts; adenocarcinoma cell lines LA-4 and MLE12), showing downregulation of c-myc expression (79%) only in integrin-rich LA-4 cells [281]. Intratracheal administration in mice bearing orthografted LA-4 tumours, showed significant suppression of tumour cells in lung tissue in comparison with non-targeted NPs, with 80% prolongation of survival time. *Ex vivo* tumour cells staining for caspase 3 and MYC protein showed downregulation of MYC and high expression of caspase 3. Tumour growth inhibition was estimated at around 80% by fluorescence in mice bearing orthotopic luciferase-expressing CMT/167 lung adenocarcinoma [281].

Experimental models using green fluorescence protein (GFP)-siRNA were set up *in vitro* [282,283]. A delivery system for MRI imaging was prepared by loading the siRNA on BSA-coated manganese-doped IONPs labelled with an RGD peptide. It was tested in MDA-MB-435-GFP cells overexpressing both integrin $\alpha_v\beta_3$ and GFP, and in $\alpha_v\beta_3$ negative A549 cells [282]. The cells were imaged by MRI and by fluorescence, showing integrin-related uptake and potency in knocking down GPF expression. The use of fluorescent quantum dots to prepare traceable GFP-siRNA delivery systems was also reported [283].

Interestingly, a study in PC3 prostate cancer cells indicates that the delivery of siRNA from $\alpha_v\beta_3$-targeted NPs [248] was reinforced by sonoporation induced by ultrasound disruption of microbubbles (see Section 7.4).

Studies targeting integrin $\alpha_5\beta_1$ have also been reported employing stealth liposomes, polyplexes, lipoplexes, and polymersomes functionalised with the PR_b peptides, and loaded with different nucleic acids, including siRNA and DNA (see Section 2.3) [75–77].

9.2. Antisense oligonucleotides delivery

Liu *et al.* prepared liposome NPs labelled with cRGDfK and loaded with anti-miR-296 antisense oligonucleotide (AON), aiming at interrupting miR-296 induced angiogenesis in endothelial cells [284]. NPs were prepared by mixing the anti-miR-296 AON with hyaluronic acid and protamine and then loading on preformed liposomes. Finally, DSPE-PEG-cRGD was added to the liposomes in 1:5 molar ratio respect DSPE-PEG. The RT-PCR analysis of miR-296 in HUVECs after 24 h incubation with targeted or non-targeted liposomes, containing either anti-miR-296 AON or a mismatch AON, revealed that the pattern of miR-296 was almost unchanged when HUVECs were treated with anti-miR-296 in non-targeted liposomes or with mismatch AON in targeted liposomes. In contrast, no miR-296 was detectable after administration of liposomes with combined anti-miR-296 and targeting. The antiangiogenic activity of integrin targeted anti-miR-296 loaded liposomes was assessed *in vitro* by the inhibition of tubules and branches formation in HUVECs on Matrigel. The results were confirmed *in vivo* by a significant decrease in microvessel formation within Matrigel plugs in mice.

Zhang *et al.* employed quantum dots (QDs) dually targeted for integrin and epidermal growth factor receptor (EGFR) to deliver anti-survivin AON to cancer cells [285]. These NPs were prepared by coating the surface of CdSe/ZnS QDs with streptavidin. Then the biotinylated AON, biotinylated EGFR targeting peptide GE11 (i.e., YHWYGYTPQNVI, identified through random peptide phage display), and biotinylated c(RGDfK) were assembled onto the surface of QDs in optimised ratios. The AON was modified with disulphide links, to be cleaved inside the cells for facilitating the release. The construct was tested *in vitro* in HeLa cells over-expressing both EGFR and $\alpha_v\beta_3$ integrin, in MDA-MB-231 cells over-expressing only EGFR, and in MCF-7 cells characterised by low expression of both receptors. Confocal imaging, flow cytometry, and ICP/MS showed that the amount of internalisation correlated with both labels, being minimal in cells poor of both receptors. The synergistic effect of dual targeting was confirmed by western blot analysis for survivin.

An important study, indicating the possible application of mAb targeting the transferrin receptor for AONs transport in brain tumours is reported (Section 8.2) [255].

10. Conclusion

The present survey does not point out substantial progress in developments towards clinical investigation and therapeutic applications in cancer and other diseases, with respect to previous reviews. However, it points out an enormous effort of innovative research, attempting to overcome the difficulties previously encountered and possibly to warrant combined efficacy and technological feasibility for competitive developments, with respect to traditional formulations and non-targeted delivery systems. In many ways, a large number of investigators, in academic institutions with worldwide distribution, have applied great ingenuity in working out novel, rationally designed approaches that may drive an active agent over the hurdles of off-target distribution and uptake, fast removal from circulation and, nearing the therapeutic target, inadequate selectivity, or lack of penetration in tissues and cells. Research was also addressed to an appropriate balancing of local retention with effective release of the active agent at the intracellular sites of action. The efforts were almost exclusively exerted in the oncological field, whereas in the field of cardiovascular diseases the work is still prevailingly addressed to diagnostic problems.

New results, promising of future development, were afforded by the search of new integrin ligands still focussing on $\alpha_v\beta_3$, the most investigated target for drug delivery, but also targeting $\alpha_v\beta_6$, $\alpha_4\beta_1$, and $\alpha_2\beta_1$ integrins, and cooperative binding sites of integrin $\alpha_5\beta_1$. Innovative design of novel integrin-targeting delivery systems, either of the prodrug type or the nanoparticulate type, with new materials and technologies, such as graphene and its oxide, or mesoporous silica also calls attention for future developments, which could also be extended from integrins to other molecular targets.

In our analysis we underscore the biological investigation that appears most promising for future clinical translation, pointing out on one side the extension from cellular experiments to *in vivo* models of cancer, and on the other the results of biodistribution studies *in vivo*. It is disappointing to admit that frequently the amount of biological information is not proportional to the chemical and technological efforts put into action, in particular when such a consideration applies to a delivery system designed and constructed with remarkable ingenuity. On a more optimistic tone, it can be thought that biological results of some of the studies, taking advantage of innovative modalities, will encourage further biological investigations with adequate resources. This would be of help in going across the no-man's-land that appear to separate academic and industrial research in the area, no longer missing the opportunity of synergism of respective research and development expertise. The lack of contribution by industrial laboratories in the discipline is of concern, considering the amount of work and resources that the pharmaceutical industry is devoting to the advancement of therapy for cancer with antibodies and with new molecules.

Innovative methodologies inviting further research work and possibly collaboration of academy and industry can be pointed out as follows:

- Combined use of cell penetrating peptides and labels for integrins, or use of the iRGD peptide, endowed with both cell penetrating and integrin targeting properties
- Devices combining integrin-targeting labels and drugs via linkers cleavable by tumour-specific mechanisms
- *In vivo* removal of a PEG cover with an S-S-link, activated by an exogenous dose of L-cysteine, in order to expose the targeting and internalising moieties
- Photothermal ablation of U87MG glioma cells by targeted photoresponsive devices, possibly transferable to intracranial surgery
- Use of "bioinspired" delivery systems, particularly ferritin, offerings room in its cage structure for organic molecules and metal ions or complexes, and also including RGDC4 labelling by genetic manipulation
- Drug loaded and gas-filled microbubbles with surface integrin labelling. Integrin affinity may induce retention in microvessels, thus allowing ultrasound imaging and disruption by sonoporation, e.g. of thrombi or the blood brain barrier, with consequent focal release and penetration of the drug.

Some of these innovative methodologies, e.g. iRGD for penetration, or coupling RGD labelling with folic acid transport, or use of microbubbles for sonoporation, have been already applied in the search of systems that could promote anticancer drug transport through the blood–brain barrier. Importantly, targeting of brain cancer could respond to the medical demand in a field where, using the word of the active investigators Ruoslahti and colleagues, "new therapies are desperately needed" [198].

Attention is also deserved, in our view, by the targeted delivery of oligonucleotides, which is now undertaken with appropriate adjustments of methodology, and appears attractive, since gene modulation could represent in the future a third arm of cancer therapy, in addition to cytotoxic agents and to antibodies. It could gain importance if modulation of gene mechanisms specific of some cancers could be coupled with selective targeting of integrins, or of other relevant molecular markers, thus affording precise delivery of adequate therapeutic doses.

Acknowledgements

D.A. gratefully acknowledge Consiglio Nazionale delle Ricerche (CNR) and Ministero dell'Universita e della Ricerca (PRIN project 2010NRREPL: Synthesis and biomedical applications of tumour-targeting peptidomimetics) for financial support.

References

[1] C.J. Avraamides, B. Garmy-Susini, J.A. Varner, Integrins in angiogenesis and lymphangiogenesis, Nat. Rev. Cancer 8 (2008) 604–617, http://dx.doi.org/10.1038/nrc2353.
[2] G.C. Alghisi, C. Rüegg, Vascular integrins in tumor angiogenesis: mediators and therapeutic targets, Endothelium 13 (2006) 113–135.
[3] J.S. Desgrosellier, D.A. Cheresh, Integrins in cancer: biological implications and therapeutic opportunities, Nat. Rev. Cancer 10 (2010) 9–22, http://dx.doi.org/10.1038/nrc2748.
[4] D. Cox, M. Brennan, N. Moran, Integrins as therapeutic targets: lessons and opportunities, Nat. Rev. Drug Discov. 9 (2010) 804–820, http://dx.doi.org/10.1038/nrd3266.
[5] M. Millard, S. Odde, N. Neamati, Integrin targeted therapeutics, Theranostics 1 (2011) 154–188.
[6] S.L. Goodman, M. Picard, Integrins as therapeutic targets, Trends Pharmacol. Sci. 33 (2012) 405–412, http://dx.doi.org/10.1016/j.tips.2012.04.002.
[7] U.K. Marelli, F. Rechenmacher, T.R. Sobahi, C. Mas-Moruno, H. Kessler, Tumor targeting via integrin ligands, Front. Oncol. 3 (2013) 222, http://dx.doi.org/10.3389/fonc.2013.00222.
[8] A.R. Reynolds, I.R. Hart, A.R. Watson, J.C. Welti, R.G. Silva, S.D. Robinson, G. Da Violante, M. Gourlaouen, M. Salih, M.C. Jones, D.T. Jones, G. Saunders, V. Kostourou, F. Perron-Sierra, J.C. Norman, G.C. Tucker, K.M. Hodivala-Dilke, Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors, Nat. Med. 15 (2009) 392–400, http://dx.doi.org/10.1038/nm.1941.
[9] S. Panzeri, S. Zanella, D. Arosio, L. Vahdati, A. Dal Corso, L. Pignataro, M. Paolillo, S. Schinelli, L. Belvisi, C. Gennari, U. Piarulli, Cyclic isoDGR and RGD peptidomimetics containing bifunctional diketopiperazine scaffolds are integrin antagonists, Chem. Eur. J. 21 (2015) 6265–6271, http://dx.doi.org/10.1002/chem.201406567.
[10] F.A. Venning, L. Wullkopf, J.T. Erler, Targeting ECM disrupts cancer progression, Front. Oncol. 5 (2015) 224, http://dx.doi.org/10.3389/fonc.2015.00224.eCollection 2015.
[11] F.L. Miles, R.A. Sikes, Insidious changes in stromal matrix fuel cancer progression, Mol. Cancer Res. 12 (2014) 297–312, http://dx.doi.org/10.1158/1541-7786.MCR-13-0535.
[12] M. Barczyk, S. Carracedo, D. Gullberg, Integrins, Cell Tissue Res. 339 (2010) 269–280, http://dx.doi.org/10.1007/s00441-009-0834-6.
[13] A. Bandyopadhyay, S. Raghavan, Defining the role of integrin alphavbeta6 in cancer, Curr. Drug Targets 10 (2009) 645–652.
[14] D. Arosio, C. Casagrande, L. Manzoni, Integrin-mediated drug delivery in cancer and cardiovascular diseases with peptide-functionalized nanoparticles, Curr. Med. Chem. 19 (2012) 3128–3151.
[15] K. Chen, X. Chen, Integrin targeted delivery of chemotherapeutics, Theranostics 1 (2011) 189–200.
[16] Z. Wang, W.K. Chui, P.C. Ho, Integrin targeted drug and gene delivery, Expert Opin. Drug Deliv. 7 (2010) 159–171, http://dx.doi.org/10.1517/17425240903468696.
[17] W. Arap, R. Pasqualini, E. Ruoslahti, Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model, Science 279 (1998) 377–380.
[18] A.G. Dupuy, E. Caron, Integrin-dependent phagocytosis: spreading from microadhesion to new concepts, J. Cell Sci. 121 (2008) 1773–1783, http://dx.doi.org/10.1242/jcs.018036.
[19] K.F. Pirollo, E.H. Chang, Does a targeting ligand influence nanoparticle tumor localization or uptake? Trends Biotechnol. 26 (2008) 552–558, http://dx.doi.org/10.1016/j.tibtech.2008.06.007.
[20] N. Nasongkla, E. Bey, J. Ren, H. Ai, C. Khemtong, J.S. Guthi, S.F. Chin, A.D. Sherry, D.A. Boothman, J. Gao, Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems, Nano Lett. 6 (2006) 2427–2430.
[21] C. Khemtong, C.W. Kessinger, J. Ren, E.A. Bey, S.G. Yang, J.S. Guthi, D.A. Boothman, A.D. Sherry, J. Gao, In vivo off-resonance saturation magnetic resonance imaging of alphavbeta3-targeted superparamagnetic nanoparticles, Cancer Res. 69 (2009) 1651–1658, http://dx.doi.org/10.1158/0008-5472.CAN-08-3231.
[22] X.B. Xiong, A. Mahmud, H. Uludağ, A. Lavasanifar, Multifunctional polymeric micelles for enhanced intracellular delivery of doxorubicin to metastatic cancer cells, Pharm. Res. 25 (2008) 2555–2566, http://dx.doi.org/10.1007/s11095-008-9673-5.
[23] H. Zhao, J.C. Wang, Q.S. Sun, C.L. Luo, Q. Zhang, RGD-based strategies for improving antitumor activity of paclitaxel-loaded liposomes in nude mice xenografted with human ovarian cancer, J. Drug Target. 17 (2009) 10–18, http://dx.doi.org/10.1080/10611860802368966.
[24] X. Jiang, H. Xin, J. Gu, X. Xu, W. Xia, S. Chen, Y. Xie, L. Chen, Y. Chen, X. Sha, X. Fang, Solid tumor penetration by integrin-mediated pegylated poly(trimethylene carbonate) nanoparticles loaded with paclitaxel, Biomaterials 34 (2013) 1739–1746, http://dx.doi.org/10.1016/j.biomaterials.2012.11.016.
[25] X. Jiang, X. Sha, H. Xin, X. Xu, J. Gu, W. Xia, S. Chen, Y. Xie, L. Chen, Y. Chen, X. Fang, Integrin-facilitated transcytosis for enhanced penetration of advanced gliomas by poly(trimethylene carbonate)-based nanoparticles encapsulating paclitaxel, Biomaterials 34 (2013) 2969–2979, http://dx.doi.org/10.1016/j.biomaterials.2012.12.049.
[26] F. Kiessling, J. Huppert, C. Zhang, J. Jayapaul, S. Zwick, E.C. Woenne, M.M. Mueller, H. Zentgraf, M. Eisenhut, Y. Addadi, M. Neeman, W. Semmler, RGD-labeled USPIO inhibits adhesion and endocytotic activity of alpha v beta3-integrin-expressing glioma cells and only accumulates in the vascular tumor compartment, Radiology 253 (2009) 462–469, http://dx.doi.org/10.1148/radiol.2532081815.
[27] C. Zhang, M. Jugold, E.C. Woenne, T. Lammers, B. Morgenstern, M.M. Mueller, H. Zentgraf, M. Bock, M. Eisenhut, W. Semmler, F. Kiessling, Specific targeting of

tumor angiogenesis by RGD-conjugated ultrasmall superparamagnetic iron oxide particles using a clinical 1.5-T magnetic resonance scanner, Cancer Res. 67 (2007) 1555–1562.
[28] B.P. Gray, M.J. McGuire, K.C. Brown, A liposomal drug platform overrides peptide ligand targeting to a cancer biomarker, irrespective of ligand affinity or density, PLoS One 8 (2013) e72938, http://dx.doi.org/10.1371/journal.pone.0072938.eCollection (2013).
[29] 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.
[30] M.R. Dreher, W. Liu, C.R. Michelich, M.W. Dewhirst, F. Yuan, A. Chilkoti, Tumor vascular permeability, accumulation, and penetration of macromolecular drug carriers, J. Natl. Cancer Inst. 98 (2006) 335–344.
[31] Z. Cheng, A. Al Zaki, J.Z. Hui, V.R. Muzykantov, A. Tsourkas, Multifunctional nanoparticles: cost versus benefit of adding targeting and imaging capabilities, Science 338 (2012) 903–910, http://dx.doi.org/10.1126/science.1226338.
[32] R. Haubner, D. Finsinger, H. Kessler, Stereoisomeric peptide libraries and peptidomimetics for designing selective inhibitors of the alpha(V)beta(3) integrin for a new cancer therapy, Angew. Chem. Int. Ed. Engl. 36 (1997) 1375–1389.
[33] M.A. Dechantsreiter, E. Planker, B. Matha, E. Lohof, G. Holzemann, A. Jonczyk, S.L. Goodmann, H. Kessler, N-methilated cyclic RGD peptides as highly active and selective alpha(V)beta(3) integrin antagonist, J. Med. Chem. 42 (1999) 3033–3040.
[34] L. Auzzas, F. Zanardi, L. Battistini, P. Burreddu, P. Carta, G. Rassu, C. Curti, G. Casiraghi, Targeting alphavbeta3 integrin: design and applications of mono- and multifunctional RGD-based peptides and semipeptides, Curr. Med. Chem. 17 (2010) 1255–1299.
[35] M. Paolillo, M.A. Russo, M. Serra, L. Colombo, S. Schinelli, Small molecule integrin antagonists in cancer therapy, Mini Rev. Med. Chem. 9 (2009) 1439–1446.
[36] X.B. Xiong, Z.S. Ma, R. Lai, A. Lavasanifar, The therapeutic response to multifunctional polymeric nano-conjugates in the targeted cellular and subcellular delivery of doxorubicin, Biomaterials 31 (2010) 757–768, http://dx.doi.org/10.1016/j.biomaterials.2009.09.080 (Erratum in: Biomaterials 32 (2011) 4194. Dosage error in article text).
[37] X.B. Xiong, H. Uludağ, A. Lavasanifar, Virus-mimetic polymeric micelles for targeted siRNA delivery, Biomaterials 31 (2010) 5886–5893, http://dx.doi.org/10.1016/j.biomaterials.2010.03.075.
[38] X.B. Xiong, A. Lavasanifar, Traceable multifunctional micellar nanocarriers for cancer-targeted co-delivery of MDR-1 siRNA and doxorubicin, ACS Nano 28 (2011) 5202–5213, http://dx.doi.org/10.1021/nn2013707.
[39] F.M. de Groot, H.J. Broxterman, H.P. Adams, A. van Vliet, G.I. Tesser, Y.W. Elderkamp, A.J. Schraa, R.J. Kok, G. Molema, H.M. Pinedo, H.W. Scheeren, Design, synthesis, and biological evaluation of a dual tumor-specific motive containing integrin-targeted plasmin-cleavable doxorubicin prodrug, Mol. Cancer Ther. 1 (2002) 901–911.
[40] H. Wang, K. Chen, W. Cai, Z. Li, L. He, A. Kashefi, X. Chen, Integrin-targeted imaging and therapy with RGD4C-TNF fusion protein, Mol. Cancer Ther. 7 (2008) 1044–1053, http://dx.doi.org/10.1158/1535-7163.MCT-07-2084.
[41] Z. Zhen, W. Tang, C. Guo, H. Chen, X. Lin, G. Liu, B. Fei, X. Chen, B. Xu, J. Xie, Ferritin nanocages to encapsulate and deliver photosensitizers for efficient photodynamic therapy against cancer, ACS Nano 7 (2013) 6988–6996, http://dx.doi.org/10.1021/nn402199g.
[42] Z. Zhen, W. Tang, H. Chen, X. Lin, T. Todd, G. Wang, T. Cowger, X. Chen, J. Xie, RGD-modified apoferritin nanoparticles for efficient drug delivery to tumors, ACS Nano 7 (2013) 4830–4837, http://dx.doi.org/10.1021/nn305791q.
[43] L. Manzoni, L. Belvisi, D. Arosio, M. Civera, M. Pilkington-Miksa, D. Potenza, A. Caprini, E.M. Araldi, E. Monferini, M. Mancino, F. Podestà, C. Scolastico, Cyclic RGD-containing functionalized azabicycloalkane peptides as potent integrin antagonists for tumor targeting, ChemMedChem 4 (2009) 615–632, http://dx.doi.org/10.1002/cmdc.200800422.
[44] D. Arosio, L. Manzoni, E.M. Araldi, A. Caprini, E. Monferini, C. Scolastico, Functionalized cyclic RGD peptidomimetics: conjugable ligands for αvβ3 receptor imaging, Bioconjug. Chem. 20 (2009) 1611–1617, http://dx.doi.org/10.1021/bc900155j.
[45] S. Lanzardo, L. Conti, C. Brioschi, M.P. Bartolomeo, D. Arosio, L. Belvisi, L. Manzoni, A. Maiocchi, F. Maisano, G. Forni, A new optical imaging probe targeting $\alpha_V\beta_3$ integrin in glioblastoma xenografts, Contrast Media Mol. Imaging 6 (2011) 449–458, http://dx.doi.org/10.1002/cmmi.444.
[46] L. Manzoni, L. Belvisi, D. Arosio, M.P. Bartolomeo, A. Bianchi, C. Brioschi, F. Buonsanti, C. Cabella, C. Casagrande, M. Civera, M. De Matteo, L. Fugazza, L. Lattuada, F. Maisano, L. Miragoli, C. Neira, M. Pilkington-Miksa, C. Scolastico, Synthesis of Gd and (68)Ga complexes in conjugation with a conformationally optimized RGD sequence as potential MRI and PET tumor-imaging probes, ChemMedChem 7 (2012) 1084–1093, http://dx.doi.org/10.1002/cmdc.201200043.
[47] L. Menichetti, C. Kusmic, D. Panetta, D. Arosio, D. Petroni, M. Matteucci, P.A. Salvatori, C. Casagrande, A. L'Abbate, L. Manzoni, MicroPET/CT imaging of $\alpha_V\beta_3$ integrin via a novel ^{68}Ga-NOTA-RGD peptidomimetic conjugate in rat myocardial infarction, Eur. J. Nucl. Med. Mol. Imaging 40 (2013) 1265–1274, http://dx.doi.org/10.1007/s00259-013-2432-9.
[48] M. Pilkington-Miksa, D. Arosio, L. Battistini, L. Belvisi, M. De Matteo, F. Vasile, P. Burreddu, P. Carta, G. Rassu, P. Perego, N. Carenini, F. Zunino, M. De Cesare, V. Castiglioni, E. Scanziani, C. Scolastico, G. Casiraghi, F. Zanardi, L. Manzoni, Design, synthesis, and biological evaluation of novel cRGD-paclitaxel conjugates for integrin-assisted drug delivery, Bioconjug. Chem. 23 (2012) 1610–1622, http://dx.doi.org/10.1021/bc300164t.
[49] A. Bianchi, D. Arosio, P. Perego, M. De Cesare, N. Carenini, N. Zaffaroni, M. De Matteo, L. Manzoni, Design, synthesis and biological evaluation of novel dimeric and tetrameric cRGD-paclitaxel conjugates for integrin-assisted drug delivery, Org. Biomol. Chem. 13 (2015) 7530–7541, http://dx.doi.org/10.1039/c5ob00497g.
[50] L. Battistini, P. Burreddu, A. Sartori, D. Arosio, L. Manzoni, L. Paduano, G. D'Errico, R. Sala, L. Reia, S. Bonomini, G. Rassu, F. Zanardi, Enhancement of the uptake and cytotoxic activity of doxorubicin in cancer cells by novel cRGD-semipeptide-anchoring liposomes, Mol. Pharm. 11 (2014) 2280–2293, http://dx.doi.org/10.1021/mp400718j.
[51] R. Colombo, M. Mingozzi, L. Belvisi, D. Arosio, U. Piarulli, N. Carenini, P. Perego, N. Zaffaroni, M. De Cesare, V. Castiglioni, E. Scanziani, C. Gennari, Synthesis and biological evaluation (in vitro and in vivo) of cyclic arginine-glycine-aspartate (RGD) peptidomimetic–paclitaxel conjugates targeting integrin αVβ3, J. Med. Chem. 55 (2012) 10460–10474, http://dx.doi.org/10.1021/jm301058f.
[52] A. Dal Corso, M. Caruso, L. Belvisi, D. Arosio, U. Piarulli, C. Albanese, F. Gasparri, A. Marsiglio, F. Sola, S. Troiani, B. Valsasina, L. Pignataro, D. Donati, C. Gennari, Synthesis and biological evaluation of RGD peptidomimetic–paclitaxel conjugates bearing lysosomally cleavable linkers, Chem. Eur. J. 21 (2015) 6921–6929, http://dx.doi.org/10.1002/chem.201500158.
[53] A. Spitaleri, S. Mari, F. Curnis, C. Traversari, R. Longhi, C. Bordignon, A. Corti, G.P. Rizzardi, G. Musco, Structural basis for the interaction of isoDGR with the RGD-binding site of alphavbeta3 integrin, J. Biol. Chem. 283 (2008) 19757–19768, http://dx.doi.org/10.1074/jbc.M710273200.
[54] L. Hou, X. Zhao, P. Wang, Q. Ning, M. Meng, C. Liu, Antitumor activity of antimicrobial peptides containing CisoDGRC in CD13 negative breast cancer cells, PLoS One 8 (2013), e53491http://dx.doi.org/10.1371/journal.pone.0053491.
[55] F. Curnis, A. Sacchi, R. Longhi, B. Colombo, A. Gasparri, A. Corti, IsoDGR-tagged albumin: a new αvβ3 selective carrier for nanodrug delivery to tumors, Small 9 (2013) 673–678.
[56] M. Pisano, I.D.E. Paola, V. Nieddu, I. Sassu, S. Cossu, G. Galleri, A. Del Gatto, M. Budroni, A. Cossu, M. Saviano, G. Palmieri, L. Zaccaro, C. Rozzo, In vitro activity of the αvβ3 integrin antagonist RGDechi-hCit on malignant melanoma cells, Anticancer Res. 33 (2013) 871–879.
[57] D. Capasso, I. de Paola, A. Liguoro, A. Del Gatto, S. Di Gaetano, D. Guarnieri, M. Saviano, L. Zaccaro, RGDechi-hCit: αvβ3 selective pro-apoptotic peptide as potential carrier for drug delivery into melanoma metastatic cells, PLoS One 9 (2014), e106441http://dx.doi.org/10.1371/journal.pone.0106441. eCollection 2014.
[58] A. Del Gatto, L. Zaccaro, P. Grieco, E. Novellino, A. Zannetti, S. Del Vecchio, F. Iommelli, M. Salvatore, C. Pedone, M. Saviano, Novel and selective alpha(v)beta3 receptor peptide antagonist: design, synthesis, and biological behaviour, J. Med. Chem. 49 (2006) 3416–3420.
[59] H. Zhang, O.H. Aina, K.S. Lam, R. de Vere White, C. Evans, P. Henderson, P.N. Lara, X. Wang, J.A. Bassuk, C.X. Pan, Identification of a bladder cancer-specific ligand using a combinatorial chemistry approach, Urol. Oncol. 30 (2012) 635–645, http://dx.doi.org/10.1016/j.urolonc.2010.06.011.
[60] T.Y. Lin, H. Zhang, J. Luo, Y. Li, T. Gao, P.N. Lara Jr., R. de Vere White, K.S. Lam, C.X. Pan, Multifunctional targeting micelle nanocarriers with both imaging and therapeutic potential for bladder cancer, Int. J. Nanomedicine 7 (2012) 2793–2804, http://dx.doi.org/10.2147/IJN.S27734.
[61] T.Y. Lin, Y.P. Li, H. Zhang, J. Luo, N. Goodwin, T. Gao, R. de Vere White, K.S. Lam, C.X. Pan, Tumor-targeting multifunctional micelles for imaging and chemotherapy of advanced bladder cancer, Nanomedicine (London) 8 (2013) 1239–1251, http://dx.doi.org/10.2217/nnm.12.150.
[62] S. Li, M.J. McGuire, M. Lin, Y.-H. Liu, T. Oyama, X. Sun, K.C. Brown, Synthesis and characterization of a high-affinity $\alpha_v\beta_6$-specific ligand for in vitro and in vivo applications, Mol. Cancer Ther. 8 (2009) 1239–1249, http://dx.doi.org/10.1158/1535-7163.MCT-08-1098.
[63] H. Guan, M.J. McGuire, S. Li, K.C. Brown, Peptide-targeted polyglutamic acid doxorubicin conjugates for the treatment of $\alpha_v\beta_6$-positive cancers, Bioconjug. Chem. 19 (2008) 1813–1821, http://dx.doi.org/10.1021/bc800154f.
[64] S. Li, B.P. Gray, M.J. McGuire, K.C. Brown, Synthesis and biological evaluation of a peptide-paclitaxel conjugate which targets the integrin $\alpha_v\beta_6$, Bioorg. Med. Chem. 19 (2011) 5480–5489, http://dx.doi.org/10.1016/j.bmc.2011.07.046.
[65] J.S. Guthi, S.-G. Yang, G. Huang, S. Li, C. Khemtong, C.W. Kessinger, M. Peyton, J.D. Minna, K.C. Brown, J. Gao, MRI-visible micellar nanomedicine for targeted drug delivery to lung cancer cells, Mol. Pharm. 7 (2010) 32–40, http://dx.doi.org/10.1021/mp9001393.
[66] B.P. Gray, S. Li, K.C. Brown, From phage display to nanoparticle delivery: functionalizing liposomes with multivalent peptides improves targeting to a cancer biomarker, Bioconjug. Chem. 24 (2013) 85–96, http://dx.doi.org/10.1021/bc300498d.
[67] F. Rechenmacher, S. Neubauer, J. Polleux, C. Mas-Moruno, M. De Simone, E.A. Cavalcanti-Adam, J.P. Spatz, R. Fässler, H. Kessler, Functionalizing αvβ3- or α5β1-selective integrin antagonists for surface coating: a method to discriminate integrin subtypes in vitro, Angew. Chem. Int. Ed. 52 (2013) 1572–1575, http://dx.doi.org/10.1002/anie.201206370.
[68] S. Neubauer, F. Rechenmacher, A.J. Beer, F. Curnis, K. Pohle, C. D'Alessandria, H.J. Wester, U. Reuning, A. Corti, M. Schwaiger, H. Kessler, Selective imaging of the angiogenic relevant integrins α5β1 and αvβ3, Angew. Chem. Int. Ed. Engl. 52 (2013) 11656–11659, http://dx.doi.org/10.1002/anie.201306376.
[69] S. Neubauer, F. Rechenmacher, R. Brimioulle, F.S. Di Leva, A. Bochen, T.R. Sobahi, M. Schottelius, E. Novellino, C. Mas-Moruno, L. Marinelli, H. Kessler, Pharmacophoric modifications lead to superpotent αvβ3 integrin ligands with suppressed α5β1 activity, J. Med. Chem. 57 (2014) 3410–3417, http://dx.doi.org/10.1021/jm500092w.
[70] W. Dai, T. Yang, X. Wang, J. Wang, X. Zhang, Q. Zhang, PHSCNK-modified and doxorubicin-loaded liposomes as a dual targeting system to integrin-overexpressing tumor neovasculature and tumor cells, J. Drug Target. 18 (2010) 254–263, http://dx.doi.org/10.3109/10611860903353354.

[71] A. Mardilovich, J.A. Craig, M.Q. McCammon, A. Garg, E. Kokkoli, Design of a novel fibronectin-mimetic peptide-amphiphile for functionalized biomaterials, Langmuir 22 (2006) 3259–3264.

[72] A. Garg, A.W. Tisdale, E. Haidari, E. Kokkoli, Targeting colon cancer cells using PEGylated liposomes modified with a fibronectin-mimetic peptide, Int. J. Pharm. 366 (2009) 201–210, http://dx.doi.org/10.1016/j.ijpharm.2008.09.016.

[73] K. Shroff, E. Kokkoli, PEGylated liposomal doxorubicin targeted to α5β1-expressing MDAMB-231 breast cancer cells, Langmuir 28 (2012) 4729–4736, http://dx.doi.org/10.1021/la204466g.

[74] M.A. Petersen, M.A. Hillmyer, E. Kokkoli, Bioresorbable polymersomes for targeted delivery of cisplatin, Bioconjug. Chem. 24 (2013) 533–543, http://dx.doi.org/10.1021/bc3003259.

[75] T.O. Pangburn, K. Georgiou, F.S. Bates, E. Kokkoli, Targeted polymersome delivery of siRNA induces cell death of breast cancer cells dependent upon Orai3 protein expression, Langmuir 28 (2012) 12816–12830, http://dx.doi.org/10.1021/la300874z.

[76] M.M. Adil, Z.S. Erdman, E. Kokkoli, Transfection mechanisms of polyplexes, lipoplexes, and stealth liposomes in α5β1 integrin bearing DLD-1 colorectal cancer cells, Langmuir 30 (2014) 3802–3810, http://dx.doi.org/10.1021/la5001396.

[77] M.M. Adil, R.M. Levine, E. Kokkoli, Increasing cancer-specific gene expression by targeting overexpressed α5β1 integrin and upregulated transcriptional activity of NF-κB, Mol. Pharm. 11 (2014) 849–858, http://dx.doi.org/10.1021/mp400535v.

[78] D. Wang, B. Fei, L.V. Halig, X. Qin, Z. Hu, H. Xu, Y.A. Wang, Z. Chen, S. Kim, D.M. Shin, Z.G. Chen, Targeted iron-oxide nanoparticle for photodynamic therapy and imaging of head and neck cancer, ACS Nano 8 (2014) 6620–6632.

[79] T. Kiziltepe, J.D. Ashley, J.F. Stefanick, Y.M. Qi, N.J. Alves, M.W. Handlogten, M.A. Suckow, R.M. Navari, B. Bilgicer, Rationally engineered nanoparticles target multiple myeloma cells, overcome cell-adhesion-mediated drug resistance, and show enhanced efficacy in vivo, Blood Cancer J. 2 (2012), e64http://dx.doi.org/10.1038/bcj.2012.10.

[80] J.F. Stefanick, J.D. Ashley, T. Kiziltepe, B. Bilgicer, A systematic analysis of peptide linker length and liposomal polyethylene glycol coating on cellular uptake of peptide-targeted liposomes, ACS Nano 23 (2013) 2935–2947, http://dx.doi.org/10.1021/nn305663e.

[81] J.F. Stefanick, J.D. Ashley, B. Bilgicer, Enhanced cellular uptake of peptide-targeted nanoparticles through increased peptide hydrophilicity and optimized ethylene glycol peptide-linker length, ACS Nano 24 (2013) 8115–8127, http://dx.doi.org/10.1021/nn4033954.

[82] W.D. Staatz, K.F. Fok, M.M. Zutter, S.P. Adams, B.A. Rodriguez, S.A. Santoro, Identification of a tetrapeptide recognition sequence for the alpha 2 beta 1 integrin in collagen, J. Biol. Chem. 266 (1991) 7363–7367.

[83] C.W. Huang, Z. Li, P.S. Conti, In vivo near-infrared fluorescence imaging of integrin α2β1 in prostate cancer with cell-penetrating-peptide-conjugated DGEA probe, J. Nucl. Med. 52 (2011) 1979–1986, http://dx.doi.org/10.2967/jnumed.111.091256.

[84] C.W. Huang, Z. Li, H. Cai, K. Chen, T. Shahinian, P.S. Conti, Design, synthesis and validation of integrin α2β1-targeted probe for microPET imaging of prostate cancer, Eur. J. Nucl. Med. Mol. Imaging 38 (2011) 1313–1322, http://dx.doi.org/10.1007/s00259-011-1752-x.

[85] A.D. Salaam, P. Hwang, R. McIntosh, H.N. Green, H.W. Jun, D. Dean, Nanodiamond-DGEA peptide conjugates for enhanced delivery of doxorubicin to prostate cancer, Beilstein J. Nanotechnol. 5 (2014) (2014) 937–945, http://dx.doi.org/10.3762/bjnano.5.107.eCollection.

[86] Y.H. Chung, P.H. Hsu, C.W. Huang, W.C. Hsieh, F.T. Huang, W.C. Chang, H. Chiu, S.T. Hsu, T.C. Yen, Evaluation of prognostic integrin α2β1 PET tracer and concurrent targeting delivery using focused ultrasound for brain glioma detection, Mol. Pharm. 11 (2014) 3904–3914, http://dx.doi.org/10.1021/mp500296n.

[87] M.W. Miller, S. Basra, D.W. Kulp, P.C. Billings, S. Choi, M.P. Beavers, O.J. McCarty, Z. Zou, M.L. Kahn, J.S. Bennett, W.F. DeGrado, Small-molecule inhibitors of integrin alpha2beta1 that prevent pathological thrombus formation via an allosteric mechanism, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 719–724, http://dx.doi.org/10.1073/pnas.0811622106.

[88] (Ivaska 1999). J. Ivaska, J. Käpylä, O. Pentikäinen, A.M. Hoffrén, J. Hermonen, P. Huttunen, M.S. Johnson, J. Heino, A peptide inhibiting the collagen binding function of integrin alpha2I domain, J. Biol. Chem. 274 (1999) 3513–3521.

[89] N.Ø. Knudsen, R.M. Schiffelers, L. Jorgensen, J. Hansen, S. Frokjaer, C. Foged, Design of cyclic RKKH peptide-conjugated PEG liposomes targeting the integrin $\alpha_2\beta_1$ receptor, Int. J. Pharm. 428 (2012) 171–177, http://dx.doi.org/10.1016/j.ijpharm.2012.02.043.

[90] P.T. Wong, S.K. Choi, Mechanisms of drug release in nanotherapeutic delivery systems, Chem. Rev. 115 (2015) 3388–3432, http://dx.doi.org/10.1021/cr5004634.

[91] J. Liu, Y. Huang, A. Kumar, A. Tan, S. Jin, A. Mozhi, X.J. Liang, pH-Sensitive nanosystems for drug delivery in cancer therapy, Biotechnol. Adv. 32 (2014) 693–710, http://dx.doi.org/10.1016/j.biotechadv.2013.11.009.

[92] A. Dal Corso, L. Pignataro, L. Belvisi, C. Gennari, $\alpha_v\beta_3$ integrin-targeted peptide/peptidomimetic-drug conjugates: in-depth analysis of the linker technology, Curr. Top. Med. Chem. (2015)http://dx.doi.org/10.2174/1568026615666150701114343.

[93] 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, http://dx.doi.org/10.1016/j.biomaterials.2014.06.042.

[94] L. Zhang, S. Zhu, L. Qian, Y. Pei, Y. Qiu, Y. Jiang, RGD-modified PEG-PAMAM-DOX conjugates: in vitro and in vivo studies for glioma, Eur. J. Pharm. Biopharm. 79 (2011) 232–240, http://dx.doi.org/10.1016/j.ejpb.2011.03.025.

[95] X. Yang, H. Hong, J.J. Grailer, I.J. Rowland, A. Javadi, S.A. Hurley, Y. Xiao, Y. Yang, Y. Zhang, R.J. Nickles, W. Cai, D.A. Steeber, S. Gong, cRGD-functionalized, DOX-conjugated, and ^{64}Cu-labeled superparamagnetic iron oxide nanoparticles for targeted anticancer drug delivery and PET/MR imaging, Biomaterials 32 (2011) 4151–4160, http://dx.doi.org/10.1016/j.biomaterials.2011.02.006.

[96] Y. Xiao, H. Hong, A. Javadi, J.W. Engle, W. Xu, Y. Yang, Y. Zhang, T.E. Barnhart, W. Cai, S. Gong, Multifunctional unimolecular micelles for cancer-targeted drug delivery and positron emission tomography imaging, Biomaterials 33 (2012) 3071–3082, http://dx.doi.org/10.1016/j.biomaterials.2011.12.030.

[97] Z.H. Peng, J. Kopeček, Enhancing accumulation and penetration of HPMA copolymer–doxorubicin conjugates in 2D and 3D prostate cancer cells via iRGD conjugation with an MMP-2 cleavable spacer, J. Am. Chem. Soc. 137 (2015) 6726–6729, http://dx.doi.org/10.1021/jacs.5b00922.

[98] D.G. Kingston, Recent advances in the chemistry of taxol, J. Nat. Prod. 63 (2000) 726–734.

[99] X. Chen, C. Plasencia, Y. Hou, N. Neamati, Synthesis and biological evaluation of dimeric RGD peptide-paclitaxel conjugate as a model for integrin-targeted drug delivery, J. Med. Chem. 48 (2005) 1098–1106 (Erratum in J. Med. Chem. 48 (2005) 5874).

[100] Q. Cao, Z.B. Li, K. Chen, Z. Wu, L. He, N. Neamati, X. Chen, Evaluation of biodistribution and anti-tumor effect of a dimeric RGD peptide-paclitaxel conjugate in mice with breast cancer, Eur. J. Nucl. Med. Mol. Imaging 35 (2008) 1489–1498, http://dx.doi.org/10.1007/s00259-008-0744-y.

[101] C. Ryppa, H. Mann-Steinberg, M.L. Biniossek, R. Satchi-Fainaro, F. Kratz, In vitro and in vivo evaluation of a paclitaxel conjugate with the divalent peptide E-[c(RGDfK)2] that targets integrin αVβ3, Int. J. Pharm. 368 (2009) 89–97, http://dx.doi.org/10.1016/j.ijpharm.2008.09.055.

[102] K.Y. Choi, M. Swierczewska, S. Lee, X. Chen, Protease-activated drug development, Theranostics 2 (2012) 156–178, http://dx.doi.org/10.7150/thno.4068.

[103] C. Ryppa, H. Mann-Steinberg, I. Fichtner, H. Weber, R. Satchi-Fainaro, M.L. Biniossek, F. Kratz, *In vitro* and *in vivo* evaluation of doxorubicin conjugates with the divalent peptide E-[c(RGDfK)$_2$] that targets integrin αvβ3, Bioconjug. Chem. 19 (2008) 1414–1422, http://dx.doi.org/10.1021/bc800117r.

[104] K.N. Sugahara, T. Teesalu, P.P. Karmali, V.R. Kotamraju, L. Agemy, O.M. Girard, D. Hanahan, R.F. Mattrey, E. Ruoslahti, Tissue-penetrating delivery of compounds and nanoparticles into tumors, Cancer Cell 16 (2009) 510–520, http://dx.doi.org/10.1016/j.ccr.2009.10.013.

[105] A. Dal Pozzo, M.H. Ni, E. Esposito, S. Dallavalle, L. Musso, A. Bargiotti, C. Pisano, L. Vesci, F. Bucci, M. Castorina, R. Foderà, G. Giannini, C. Aulicino, S. Penco, Novel tumor-targeted RGD peptide-camptothecin conjugates: synthesis and biological evaluation, Bioorg. Med. Chem. 18 (2010) 64–72, http://dx.doi.org/10.1016/j.bmc.2009.11.019.

[106] A. Dal Pozzo, E. Esposito, M. Ni, L. Muzi, C. Pisano, F. Bucci, L. Vesci, M. Castorina, S. Penco, Conjugates of a novel 7-substituted camptothecin with RGD-peptides as α(v)β3 integrin ligands: an approach to tumor-targeted therapy, Bioconjug. Chem. 21 (2010) 1956–1967, http://dx.doi.org/10.1021/bc100097r.

[107] M.H. Wu, B. Yan, R. Humerickhouse, M.E. Dolan, Irinotecan activation by human carboxylesterases in colorectal adenocarcinoma cells, Clin. Cancer Res. 8 (2002) 2696–2700.

[108] S. Iwama, T. Kitano, F. Fukuya, Y. Honda, Y. Sato, M. Notako, T. Morie, Discovery of a potent and selective alpha v beta 3 integrin antagonist with strong inhibitory activity against neointima formation in rat balloon injury model, Bioorg. Med. Chem. Lett. 14 (2004) 2567–2570.

[109] D. Alloatti, G. Giannini, L. Vesci, M. Castorina, C. Pisano, E. Badaloni, W. Cabri, Camptothecins in tumor homing via an RGD sequence mimetic, Bioorg. Med. Chem. Lett. 22 (2012) 6509–6512, http://dx.doi.org/10.1016/j.bmcl.2012.07.061.

[110] M.H. Lee, J.Y. Kim, J.H. Han, S. Bhuniya, J.L. Sessler, C. Kang, J.S. Kim, Direct fluorescence monitoring of the delivery and cellular uptake of a cancer-targeted RGD peptide-appended naphthalimide theragnostic prodrug, J. Am. Chem. Soc. 134 (2012) 12668–12674, http://dx.doi.org/10.1021/ja303998y.

[111] N. Graf, S.J. Lippard, Redox activation of metal-based prodrugs as a strategy for drug delivery, Adv. Drug Deliv. Rev. 64 (2012) 993–1004, http://dx.doi.org/10.1016/j.addr.2012.01.007.

[112] S. Mukhopadhyay, C.M. Barnés, A. Haskel, S.M. Short, K.R. Barnes, S.J. Lippard, Conjugated platinum(IV)-peptide complexes for targeting angiogenic tumor vasculature, Bioconjug. Chem. 19 (2008) 39–49.

[113] N. Graf, D.R. Bielenberg, N. Kolishetti, C. Muus, J. Banyard, O.C. Farokhzad, S.J. Lippard, α(V)β(3) integrin-targeted PLGA-PEG nanoparticles for enhanced anti-tumor efficacy of a Pt(IV) prodrug, ACS Nano 6 (2012) 4530–4539, http://dx.doi.org/10.1021/nn301148e.

[114] W.J. Rieter, K.M. Pott, K.M.L. Taylor, W. Lin, Nanoscale coordination polymers for platinum-based anticancer drug delivery, J. Am. Chem. Soc. 130 (2008) 11584–11585.

[115] K.M.L. Taylor-Pashow, J. Della Rocca, Z. Xie, S. Tran, W. Lin, Postsynthetic modifications of iron-carboxylate nanoscale metal–organic frameworks for imaging and drug delivery, J. Am. Chem. Soc. 131 (2009) 14261–14263.

[116] Y. Yuan, R.T. Kwok, B.Z. Tang, B. Liu, Targeted theranostic platinum(IV) prodrug with a built-in aggregation-induced emission light-up apoptosis sensor for noninvasive early evaluation of its therapeutic responses in situ, J. Am. Chem. Soc. 136 (2014) 2546–2554, http://dx.doi.org/10.1021/ja411811w.

[117] Y. Yuan, R.T. Kwok, R. Zhang, B.Z. Tang, B. Liu, Targeted theranostic prodrugs based on an aggregation-induced emission (AIE) luminogen for real-time dual-drug tracking, Chem. Commun. 50 (2014) 11465–11468, http://dx.doi.org/10.1039/c4cc05255b.

[118] A. Massaguer, A. González-Cantó, E. Escribano, S. Barrabés, G. Artigas, V. Moreno, V. Marchán, Integrin-targeted delivery into cancer cells of a Pt(IV) pro-drug through conjugation to RGD-containing peptides, Dalton Trans. 44 (2015) 202–212, http://dx.doi.org/10.1039/c4dt02710h.

[119] D. Boturyn, E. Defrancq, G.T. Dolphin, J. Garcia, P. Labbe, O. Renaudet, P. Dumy, RAFTNano-constructs: surfing to biological applications, J. Pept. Sci. 14 (2008) 224–240.

[120] L. Sancey, E. Garanger, S. Foillard, G. Schoehn, A. Hurbin, C. Albiges-Rizo, D. Boturyn, C. Souchier, A. Grichine, P. Dumy, J.L. Coll, Clustering and internalization of integrin alphavbeta3 with a tetrameric RGD-synthetic peptide, Mol. Ther. 17 (2009) 837–843, http://dx.doi.org/10.1038/mt.2009.29.
[121] L. Sancey, V. Ardisson, L.M. Riou, M. Ahmadi, D. Marti-Batlle, D. Boturyn, P. Dumy, D. Fagret, C. Ghezzi, J.P. Vuillez, In vivo imaging of tumour angiogenesis in mice with the alpha(v)beta (3) integrin-targeted tracer 99mTc-RAFT-RGD, Eur. J. Nucl. Med. Mol. Imaging 34 (2007) 2037–2047.
[122] M. Galibert, Z.H. Jin, T. Furukawa, T. Fukumura, T. Saga, Y. Fujibayashi, P. Dumy, D. Boturyn, RGD-cyclam conjugate: synthesis and potential application for positron emission tomography, Bioorg. Med. Chem. Lett. 20 (2010) 5422–5425, http://dx.doi.org/10.1016/j.bmcl.2010.07.114.
[123] S. Dufort, L. Sancey, A. Hurbin, S. Foillard, D. Boturyn, P. Dumy, J.L. Coll, Targeted delivery of a proapoptotic peptide to tumors in vivo, J. Drug Target. 19 (2011) 582–588, http://dx.doi.org/10.3109/1061186X.2010.542245.
[124] S. Foillard, L. Sancey, J.L. Coll, D. Boturyn, P. Dumy, Targeted delivery of activatable fluorescent pro-apoptotic peptide into live cells, Org. Biomol. Chem. 21 (2009) 221–224, http://dx.doi.org/10.1039/b817251j.
[125] P.M. Winter, A.M. Neubauer, S.D. Caruthers, T.D. Harris, J.D. Robertson, T.A. Williams, A.H. Schmieder, G. Hu, J.S. Allen, E.K. Lacy, H. Zhang, S.A. Wickline, G.M. Lanza, Endothelial alpha(v)beta3 integrin-targeted fumagillin nanoparticles inhibit angiogenesis in atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 2103–2109.
[126] A.H. Schmieder, S.D. Caruthers, H. Zhang, T.A. Williams, J.D. Robertson, S.A. Wickline, G.M. Lanza, Three-dimensional MR mapping of angiogenesis with alpha5beta1(alpha nu beta3)-targeted theranostic nanoparticles in the MDAMB-435 xenograft mouse model, FASEB J. 22 (2008) 4179–4189, http://dx.doi.org/10.1096/fj.08-112060.
[127] P.M. Winter, S.D. Caruthers, H. Zhang, T.A. Williams, S.A. Wickline, G.M. Lanza, Antiangiogenic synergism of integrin-targeted fumagillin nanoparticles and atorvastatin in atherosclerosis, JACC Cardiovasc. Imaging 1 (2008) 624–634, http://dx.doi.org/10.1016/j.jcmg.2008.06.003.
[128] P.M. Winter, A.H. Schmieder, S.D. Caruthers, J.L. Keene, H. Zhang, S.A. Wickline, G.M. Lanza, Minute dosages of alpha(nu)beta3-targeted fumagillin nanoparticles impair Vx-2 tumor angiogenesis and development in rabbits, FASEB J. 22 (2008) 2758–2767, http://dx.doi.org/10.1096/fj.07-103929.
[129] H.F. Zhou, H.W. Chan, S.A. Wickline, G.M. Lanza, C.T. Pham, Alphavbeta3-targeted nanotherapy suppresses inflammatory arthritis in mice, FASEB J. 23 (2009) 2978–2985, http://dx.doi.org/10.1096/fj.09-129874.
[130] H.F. Zhou, G. Hu, S.A. Wickline, G.M. Lanza, C.T. Pham, Synergistic effect of antiangiogenic nanotherapy combined with methotrexate in the treatment of experimental inflammatory arthritis, Nanomedicine (London) 5 (2010) 1065–1074, http://dx.doi.org/10.2217/nnm.10.78.
[131] D. Pan, N. Sanyal, A.H. Schmieder, A. Senpan, B. Kim, X. Yang, G. Hu, J.S. Allen, R.W. Gross, S.A. Wickline, G.M. Lanza, Anti-angiogenic nanotherapy with lipase-labile Sn-2 fumagillin prodrug, Nanomedicine (London) 7 (2012) 1507–1519, http://dx.doi.org/10.2217/nnm.12.27.
[132] H.F. Zhou, H. Yan, A. Senpan, S.A. Wickline, D. Pan, G.M. Lanza, C.T.N. Pham, Suppression of inflammation in a mouse model of rheumatoid arthritis using targeted lipase-labile fumagillin prodrug nanoparticles, Biomaterials 33 (2012) 8632–8640, http://dx.doi.org/10.1016/j.biomaterials.
[133] 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, http://dx.doi.org/10.1038/nnano.2007.387.
[134] R. van der Meel, L.J. Vehmeijer, R.J. Kok, G. Storm, E.V. van Gaal, Ligand-targeted particulate nanomedicines undergoing clinical evaluation: current status, Adv. Drug Deliv. Rev. 65 (2013) 1284–1298, http://dx.doi.org/10.1016/j.addr.2013.08.012.
[135] M. Harries, P. Ellis, P. Harper, Nanoparticle albumin-bound paclitaxel for metastatic breast cancer, J. Clin. Oncol. 23 (2005) 7768–7771.
[136] Y. Barenholz, Doxil®—the first FDA-approved nano-drug: lessons learned, J. Control. Release 160 (2012) 117–134, http://dx.doi.org/10.1016/j.jconrel.2012.03.020.
[137] L. Harris, G. Batist, R. Belt, D. Rovira, R. Navari, N. Azarnia, L. Welles, E. Winer, TLC D-99 Study Group, Liposome-encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma, Cancer 94 (2002) 25–36.
[138] R.S. Edwards, K.S. Coleman, Graphene synthesis: relationship to applications, Nanoscale 5 (2013) 38–51, http://dx.doi.org/10.1039/c2nr32629a.
[139] K. Yang, L. Feng, X. Shi, Z. Liu, Nano-graphene in biomedicine: theranostic applications, Chem. Soc. Rev. 42 (2013) 530–547, http://dx.doi.org/10.1039/c2cs35342c.
[140] J. Liu, L. Cui, D. Losic, Graphene and graphene oxide as new nanocarriers for drug delivery applications, Acta Biomater. 9 (2013) 9243–9257, http://dx.doi.org/10.1016/j.actbio.2013.08.016.
[141] J.T. Robinson, S.M. Tabakman, Y. Liang, H. Wang, H.S. Casalongue, D. Vinh, H. Dai, Ultrasmall reduced graphene oxide with high near-infrared absorbance for photothermal therapy, J. Am. Chem. Soc. 133 (2011) 6825–6831, http://dx.doi.org/10.1021/ja2010175.
[142] C. Wang, B. Chen, M. Zou, G. Cheng, Cyclic RGD-modified chitosan/graphene oxide polymers for drug delivery and cellular imaging, Colloids Surf. B: Biointerfaces 122 (2014) 332–340, http://dx.doi.org/10.1016/j.colsurfb.2014.07.018.
[143] T. Zhou, X. Zhou, D. Xing, Controlled release of doxorubicin from graphene oxide based charge-reversal nanocarriers, Biomaterials 35 (2014) 4185–4194, http://dx.doi.org/10.1016/j.biomaterials.2014.01.044.
[144] D. Chen, C.A. Dougherty, K. Zhu, H. Hong, Theranostic applications of carbon nanomaterials in cancer: focus on imaging and cargo delivery, J. Control. Release 210 (2015) 230–245, http://dx.doi.org/10.1016/j.jconrel.2015.04.021.
[145] S.H. Wu, C.Y. Mou, H.P. Lin, Synthesis of mesoporous silica nanoparticles, Chem. Soc. Rev. 42 (2013) 3862–3875, http://dx.doi.org/10.1039/c3cs35405a.
[146] Y. Wang, Q. Zhao, N. Han, L. Bai, J. Li, J. Liu, E. Che, L. Hu, Q. Zhang, T. Jiang, S. Wang, Mesoporous silica nanoparticles in drug delivery and biomedical applications, Nanomedicine 11 (2015) 313–327, http://dx.doi.org/10.1016/j.nano.2014.09.014.
[147] V. Mamaeva, C. Sahlgren, M. Lindén, Mesoporous silica nanoparticles in medicin—recent advances, Adv. Drug Deliv. Rev. 65 (2013) 689–702, http://dx.doi.org/10.1016/j.addr.2012.07.018.
[148] P. Yang, S. Gai, J. Lin, Functionalized mesoporous silica materials for controlled drug delivery, Chem. Soc. Rev. 41 (2012) 3679–3698, http://dx.doi.org/10.1039/c2cs15308d.
[149] D.P. Ferris, J. Lu, C. Gothard, R. Yanes, C.R. Thomas, J.C. Olsen, J.F. Stoddart, F. Tamanoi, J.I. Zink, Synthesis of biomolecule-modified mesoporous silica nanoparticles for targeted hydrophobic drug delivery to cancer cells, Small 7 (2011) 1816–1826, http://dx.doi.org/10.1002/smll.201002300.
[150] L. Pan, J. Liu, Q. He, J. Shi, MSN-mediated sequential vascular-to-cell nuclear-targeted drug delivery for efficient tumor regression, Adv. Mater. 26 (2014) 6742–6748, http://dx.doi.org/10.1002/adma.201402752.
[151] J. Zhang, Z.F. Yuan, Y. Wang, W.-H. Chen, G.F. Luo, S.X. Cheng, R.-X. Zhuo, X.Z. Zhang, Multifunctional envelope-type mesoporous silica nanoparticles for tumor-triggered targeting drug delivery, J. Am. Chem. Soc. 135 (2013) 5068–5073, http://dx.doi.org/10.1021/ja312004m.
[152] H. Chen, Z. Zhen, W. Tang, T. Todd, Y.J. Chuang, L. Wang, Z. Pan, J. Xie, Label-free luminescent mesoporous silica nanoparticles for imaging and drug delivery, Theranostics 3 (2013) 650–657, http://dx.doi.org/10.7150/thno.6668 (eCollection 2013).
[153] Y. Zhu, H.S. Sundaram, S. Liu, L. Zhang, X. Xu, Q. Yu, J. Xu, S. Jiang, A robust graft-to strategy to form multifunctional and stealth zwitterionic polymer-coated mesoporous silica nanoparticles, Biomacromolecules 15 (2014) 1845–1851, http://dx.doi.org/10.1021/bm500209a.
[154] Y.T. Liao, K.C.W. Wu, J. Yu, Synthesis of mesoporous silica nanoparticle-encapsulated alginate microparticles for sustained release and targeting therapy, J. Biomed. Mater. Res. B Appl. Biomater. 102 (2014) 293–302, http://dx.doi.org/10.1002/jbm.b.33007.
[155] Y.T. Liao, C.H. Liu, J. Yu, K.C.W. Wu, Liver cancer cells: targeting and prolonged-release drug carriers consisting of mesoporous silica nanoparticles and alginate microspheres, Int. J. Nanomedicine 5 (2014) 2767–2778, http://dx.doi.org/10.2147/IJN.S60171. eCollection 2014.
[156] C.F. Wang, E.M. Mäkilä, M.H. Kaasalainen, D. Liu, M.P. Sarparanta, A.J. Airaksinen, J.J. Salonen, J.T. Hirvonen, H.A. Santos, Copper-free azide–alkyne cycloaddition of targeting peptides to porous silicon nanoparticles for intracellular drug uptake, Biomaterials 35 (2014) 1257–1266, http://dx.doi.org/10.1016/j.biomaterials.2013.10.065.
[157] A. Hansson, T. Di Francesco, F. Falson, P. Rousselle, O. Jordan, G. Borchard, Preparation and evaluation of nanoparticles for directed tissue engineering, Int. J. Pharm. 439 (2012) 73–80, http://dx.doi.org/10.1016/j.ijpharm.2012.09.053.
[158] P.H. Chua, K.G. Neoh, E.T. Kang, W. Wang, Surface functionalization of titanium with hyaluronic acid/chitosan polyelectrolyte multilayers and RGD for promoting osteoblast functions and inhibiting bacterial adhesion, Biomaterials 29 (2008) 1412–1421, http://dx.doi.org/10.1016/j.biomaterials.2007.12.019.
[159] J.Y. Lee, J.E. Choo, Y.S. Choi, I.K. Shim, S.J. Lee, Y.J. Seol, C.P. Chung, Y.J. Park, Effect of immobilized cell-binding peptides on chitosan membranes for osteoblastic differentiation of mesenchymal stem cells, Biotechnol. Appl. Biochem. 52 (2009) 69–77, http://dx.doi.org/10.1042/BA20070169.
[160] M. Mochizuki, N. Yamagata, D. Philp, K. Hozumi, T. Watanabe, Y. Kikkawa, Y. Kadoya, H.K. Kleinman, M. Nomizu, Integrin-dependent cell behavior on ECM peptide-conjugated chitosan membranes, Biopolymers 88 (2007) 122–130.
[161] J.H. Kim, Y.S. Kim, K. Park, E. Kang, S. Lee, H.Y. Nam, K. Kim, J.H. Park, D.Y. Chi, R.W. Park, I.S. Kim, K. Choi, I. Chan Kwon, Self-assembled glycol chitosan nanoparticles for the sustained and prolonged delivery of antiangiogenic small peptide drugs in cancer therapy, Biomaterials 29 (2008) 1920–1930, http://dx.doi.org/10.1016/j.biomaterials.2007.12.038.
[162] L.L. Cai, P. Liu, X. Li, X. Huang, Y.Q. Ye, F.Y. Chen, H. Yuan, F.Q. Hu, Y.Z. Du, RGD peptide-mediated chitosan-based polymeric micelles targeting delivery for integrin-overexpressing tumor cells, Int. J. Nanomedicine 6 (2011) 3499–3508, http://dx.doi.org/10.2147/IJN.S26670.
[163] F.Q. Hu, P. Meng, Y.Q. Dai, Y.Z. Du, J. You, X.H. Wei, H. Yuan, PEGylated chitosan-based polymer micelle as an intracellular delivery carrier for anti-tumor targeting therapy, Eur. J. Pharm. Biopharm. 70 (2008) 749–757, http://dx.doi.org/10.1016/j.ejpb.2008.06.015.
[164] P.P. Lv, Y.F. Ma, R. Yu, H. Yue, D.Z. Ni, W. Wei, G.H. Ma, Targeted delivery of insoluble cargo (paclitaxel) by PEGylated chitosan nanoparticles grafted with Arg-Gly-Asp (RGD), Mol. Pharm. 4 (2012) 1736–1747, http://dx.doi.org/10.1021/mp300051h.
[165] H.D. Han, L.S. Mangala, J.W. Lee, M.M. Shahzad, H.S. Kim, D. Shen, E.J. Nam, E.M. Mora, R.L. Stone, C. Lu, S.J. Lee, J.W. Roh, A.M. Nick, G. Lopez-Berestein, A.K. Sood, Targeted gene silencing using RGD-labeled chitosan nanoparticles, Clin. Cancer Res. 16 (2010) 3910–3922, http://dx.doi.org/10.1158/1078-0432.CCR-10-0005.
[166] C. Alvarez-Lorenzo, A. Concheiro, Bioinspired drug delivery systems, Curr. Opin. Biotechnol. 24 (2013) 1167–1173, http://dx.doi.org/10.1016/j.copbio.2013.02.013.
[167] Z. Zhen, W. Tang, T. Todd, J. Xie, Ferritins as nanoplatforms for imaging and drug delivery, Expert Opin. Drug Deliv. 11 (2014) 1913–1922, http://dx.doi.org/10.1517/17425247.2014.941354.
[168] M. Uchida, M.L. Flenniken, M. Allen, D.A. Willits, B.E. Crowley, S. Brumfield, A.F. Willis, L. Jackiw, M. Jutila, M.J. Young, T. Douglas, Targeting of cancer cells with ferrimagnetic ferritin cage nanoparticles, J. Am. Chem. Soc. 128 (2006) 16626–16633.

[169] X. Lin, J. Xie, G. Niu, F. Zhang, H. Gao, M. Yang, Q. Quan, M.A. Aronova, G. Zhang, S. Lee, R. Leapman, X. Chen, Chimeric ferritin nanocages for multiple function loading and multimodal imaging, Nano Lett. 11 (2011) 814–819, http://dx.doi.org/10.1021/nl104141g.

[170] E. Arslan, I.C. Garip, G. Gulseren, A.B. Tekinay, M.O. Guler, Bioactive supramolecular peptide nanofibers for regenerative medicine, Adv. Healthc. Mater. 3 (2014) 1357–1376, http://dx.doi.org/10.1002/adhm.201300491.

[171] S. Soukasene, D.J. Toft, T.J. Moyer, H. Lu, H.K. Lee, S.M. Standley, V.L. Cryns, S.I. Stupp, Antitumor activity of peptide amphiphile nanofiber-encapsulated camptothecin, ACS Nano 5 (2011) 9113–9121, http://dx.doi.org/10.1021/nn203343z.

[172] P. Zhang, A.G. Cheetham, Y.A. Lin, H. Cui, Self-assembled Tat nanofibers as effective drug carrier and transporter, ACS Nano 7 (2013) 5965–5977, http://dx.doi.org/10.1021/nn401667z.

[173] J. Liu, J. Liu, H. Xu, Y. Zhang, L. Chu, Q. Liu, N. Song, C. Yang, Novel tumor-targeting, self-assembling peptide nanofiber as a carrier for effective curcumin delivery, Int. J. Nanomedicine 9 (2014) 197–207, http://dx.doi.org/10.2147/IJN.S55875.

[174] M.B. Browning, V. Guiza, B. Russell, J. Rivera, S. Cereceres, M. Höök, M.S. Hahn, E.M. Cosgriff-Hernandez, Endothelial cell response to chemical, biological, and physical cues in bioactive hydrogels, Tissue Eng. A 20 (2014) 3130–3141, http://dx.doi.org/10.1089/ten.TEA.2013.0602.

[175] M.B. Browning, B. Russell, J. Rivera, M. Höök, E.M. Cosgriff-Hernandez, Bioactive hydrogels with enhanced initial and sustained cell interactions, Biomacromolecules 14 (2013) 2225–2233, http://dx.doi.org/10.1021/bm400634j.

[176] S. Hamaia, R.W. Farndale, Integrin recognition motifs in the human collagens, Adv. Exp. Med. Biol. 819 (2014) 127–142, http://dx.doi.org/10.1007/978-94-017-9153-3_9.

[177] G. Caracciolo, F. Cardarelli, D. Pozzi, F. Salomone, G. Maccari, G. Bardi, A.L. Capriotti, C. Cavaliere, M. Papi, A. Laganà, Selective targeting capability acquired with a protein corona adsorbed on the surface of 1,2-dioleoyl-3-trimethylammonium propane/DNA nanoparticles, ACS Appl. Mater. Interfaces 26 (2013) 13171–13179, http://dx.doi.org/10.1021/am404171h.

[178] G. Caracciolo, The protein corona effect for targeted drug delivery, Bioinspired Biomimetic Nanobiomater. 2 (2012) 54–57.

[179] F. Kratz, Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles, J. Control. Release 132 (2008) 171–183, http://dx.doi.org/10.1016/j.jconrel.2008.05.010.

[180] F. Kratz, B. Elsadek, Clinical impact of serum proteins on drug delivery, J. Control. Release 161 (2012) 429–445, http://dx.doi.org/10.1016/j.jconrel.2011.11.028.

[181] B. Elsadek, F. Kratz, Impact of albumin on drug delivery—new applications on the horizon, J. Control. Release 157 (2012) 4–28, http://dx.doi.org/10.1016/j.jconrel.2011.09.069.

[182] X. Ming, K. Carver, L. Wu, Albumin-based nanoconjugates for targeted delivery of therapeutic oligonucleotides, Biomaterials 34 (2013) 7939–7949, http://dx.doi.org/10.1016/j.biomaterials.2013.06.066.

[183] H.B. Pang, G.B. Braun, Z.G. She, V.R. Kotamraju, K.N. Sugahara, T. Teesalu, E. Ruoslahti, A free cysteine prolongs the half-life of a homing peptide and improves its tumor-penetrating activity, J. Control. Release 175 (2014) 48–53, http://dx.doi.org/10.1016/j.jconrel.2013.12.006.

[184] S. Su, H. Wang, X. Liu, Y. Wu, G. Nie, iRGD-coupled responsive fluorescent nanogel for targeted drug delivery, Biomaterials 34 (2013) 3523–3533, http://dx.doi.org/10.1016/j.biomaterials.2013.01.083.

[185] Q. Chen, X. Wang, C. Wang, L. Feng, Y. Li, Z. Liu, Drug-induced self-assembly of modified albumins as nano-theranostics for tumor-targeted combination therapy, ACS Nano 9 (2015) 5223–5233, http://dx.doi.org/10.1021/acsnano.5b00640.

[186] Y. Tian, S. Li, J. Song, T. Ji, M. Zhu, G.J. Anderson, J. Wei, G. Nie, A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy, Biomaterials 35 (2014) 2383–2390, http://dx.doi.org/10.1016/j.biomaterials.2013.11.083.

[187] X. Fu, A. Rivera, L. Tao, X. Zhang, Genetically modified T cells targeting neovasculature efficiently destroy tumor blood vessels, shrink established solid tumors and increase nanoparticle delivery, Int. J. Cancer 133 (2013) 2483–2492, http://dx.doi.org/10.1002/ijc.28269.

[188] S.S. Dasa, Q. Jin, C.T. Chen, L. Chen, Target-specific copper hybrid T7 phage particles, Langmuir 28 (2012) 17372–17380, http://dx.doi.org/10.1021/la3024919.

[189] M.C. Morris, P. Vidal, L. Chaloin, F. Heitz, G. Divita, A new peptide vector for efficient delivery of oligonucleotides into mammalian cells, Nucleic Acids Res. 25 (1997) 2730–2736.

[190] F. Heitz, M.C. Morris, G. Divita, Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics, Br. J. Pharmacol. 157 (2009) 195–206, http://dx.doi.org/10.1111/j.1476-5381.2009.00057.x.

[191] W.L. Munyendo, H. Lv, H. Benza-Ingoula, L.D. Baraza, J. Zhou, Cell penetrating peptides in the delivery of biopharmaceuticals, Biomolecules 2 (2012) 187–202, http://dx.doi.org/10.3390/biom2020187.

[192] L. Mei, L. Fu, K. Shi, Q. Zhang, Y. Liu, J. Tang, H. Gao, Z. Zhang, Q. He, Increased tumor targeted delivery using a multistage liposome system functionalized with RGD, TAT and cleavable PEG, Int. J. Pharm. 468 (2014) 26–38, http://dx.doi.org/10.1016/j.ijpharm.2014.04.008.

[193] A.A. Mokhtarieh, S. Kim, Y. Lee, B.H. Chung, M.K. Lee, Novel cell penetrating peptides with multiple motifs composed of RGD and its analogs, Biochem. Biophys. Res. Commun. 432 (2013) 359–364, http://dx.doi.org/10.1016/j.bbrc.2013.01.096.

[194] J. Liang, W.L. Wu, X.D. Xu, R.X. Zhuo, X.Z. Zhang, pH responsive micelle self-assembled from a new amphiphilic peptide as anti-tumor drug carrier, Colloids Surf. B: Biointerfaces 114 (2014) 398–403, http://dx.doi.org/10.1016/j.colsurfb.2013.10.037.

[195] A.A. Mokhtarieh, S. Cheong, S. Kim, B.H. Chung, M.K. Lee, Asymmetric liposome particles with highly efficient encapsulation of siRNA and without non-specific cell penetration suitable for target-specific delivery, Biochim. Biophys. Acta 1818 (2012) 1633–1641.

[196] G.J. Prud'homme, Y. Glinka, Neuropilins are multifunctional coreceptors involved in tumor initiation, growth, metastasis and immunity, Oncotarget 3 (2012) 921–939.

[197] K.N. Sugahara, T. Teesalu, P.P. Karmali, V.R. Kotamraju, L. Agemy, D.R. Greenwald, E. Ruoslahti, Coadministration of a tumor-penetrating peptide enhances the efficacy of cancer drugs, Science 328 (5981) (May 21 2010) 1031–1035, http://dx.doi.org/10.1126/science.1183057.

[198] L. Agemy, D. Friedmann-Morvinski, V.R. Kotamraju, L. Roth, K.N. Sugahara, O.M. Girard, R.F. Mattrey, I.M. Verma, E. Ruoslahti, Targeted nanoparticle enhanced proapoptotic peptide as potential therapy for glioblastoma, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 17450–17455, http://dx.doi.org/10.1073/pnas.1114518108 (Erratum in: Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 11906).

[199] J.H. Park, G. von Maltzahn, L. Zhang, A.M. Derfus, D. Simberg, T.J. Harris, E. Ruoslahti, S.N. Bhatia, M.J. Sailor, Systematic surface engineering of magnetic nanoworms for in vivo tumor targeting, Small 5 (2009) 694–700, http://dx.doi.org/10.1002/smll.200801789.

[200] Y.L. Wu, J. Huang, J. Xu, J. Liu, Z. Feng, Y. Wang, Y. Lai, Z.R. Wu, Addition of acysteine to glucagon-like peptide-1 (GLP-1) conjugates GLP-1 to albumin in serum and prolongs GLP-1 action in vivo, Regul. Pept. 164 (2010) 83–89, http://dx.doi.org/10.1016/j.regpep.2010.05.003.

[201] R. Chen, G.B. Braun, X. Luo, K.N. Sugahara, T. Teesalu, E. Ruoslahti, Application of a proapoptotic peptide to intratumorally spreading cancer therapy, Cancer Res. 73 (2013) 1352–1361, http://dx.doi.org/10.1158/0008-5472.CAN-12-1979.

[202] W. Dai, Y. Fan, H. Zhang, X. Wang, Q. Zhang, X. Wang, A comprehensive study of iRGD-modified liposomes with improved chemotherapeutic efficacy on B16 melanoma, Drug Deliv. 22 (2015) 10–20, http://dx.doi.org/10.3109/10717544.2014.903580.

[203] K.F. Yu, W.Q. Zhang, L.M. Luo, P. Song, D. Li, R. Du, W. Ren, D. Huang, W.L. Lu, X. Zhang, Q. Zhang, The antitumor activity of a doxorubicin loaded, iRGD-modified sterically-stabilized liposome on B16-F10 melanoma cells: in vitro and in vivo evaluation, Int. J. Nanomedicine 8 (2013) 2473–2485, http://dx.doi.org/10.2147/IJN.S46962.

[204] R. Du, T. Zhong, W.Q. Zhang, P. Song, W.D. Song, Y. Zhao, C. Wang, Y.Q. Tang, X. Zhang, Q. Zhang, Antitumor effect of iRGD-modified liposomes containing conjugated linoleic acid-paclitaxel (CLA-PTX) on B16-F10 melanoma, Int. J. Nanomedicine 9 (2014) 3091–3105, http://dx.doi.org/10.2147/IJN.S65664 (eCollection 2014).

[205] L. Alberici, L. Roth, K.N. Sugahara, L. Agemy, V.R. Kotamraju, T. Teesalu, C. Bordignon, C. Traversari, G.P. Rizzardi, E. Ruoslahti, De novo design of a tumor-penetrating peptide, Cancer Res. 73 (2013) 804–812, http://dx.doi.org/10.1158/0008-5472.CAN-12-1668.

[206] A. Corti, F. Pastorino, F. Curnis, W. Arap, M. Ponzoni, R. Pasqualini, Targeted drug delivery and penetration into solid tumors, Med. Res. Rev. 32 (2012) 1078–1091, http://dx.doi.org/10.1002/med.20238.

[207] R.E. Wang, Y. Niu, H. Wu, Y. Hu, J. Cai, Development of NGR-based anti-cancer agents for targeted therapeutics and imaging, Anti Cancer Agents Med. Chem. 12 (2012) 76–86.

[208] H.M. Ellerby, D.E. Bredesen, S. Fujimura, V. John, Hunter-killer peptide (HKP) for targeted therapy, J. Med. Chem. 51 (2008) 5887–5892, http://dx.doi.org/10.1021/jm800495u (Erratum in: J. Med. Chem. 58 (2015) 2045).

[209] S. Mura, J. Nicolas, P. Couvreur, Stimuli-responsive nanocarriers for drug delivery, Nat. Mater. 12 (2013) 991–1003, http://dx.doi.org/10.1038/nmat3776.

[210] 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, http://dx.doi.org/10.1039/c2cs15344k.

[211] Z. Ge, S. Liu, Functional block copolymer assemblies responsive to tumor and intracellular microenvironments for site-specific drug delivery and enhanced imaging performance, Chem. Soc. Rev. 42 (2013) 7289–7325, http://dx.doi.org/10.1039/c3cs60048c.

[212] L. Zhu, V.P. Torchilin, Stimulus-responsive nanopreparations for tumor targeting, Integr. Biol. (Camb.) 5 (2013) 96–107, http://dx.doi.org/10.1039/c2ib20135f.

[213] M.P. Melancon, C. Li, Multifunctional synthetic poly(L-glutamic acid)-based cancer therapeutic and imaging agents, Mol. Imaging 10 (2011) 28–42.

[214] M. Haas, F. Moolenaar, A. Elsinga, E.A. Van der Wouden, P.E. de Jong, D.K. Meijer, D. de Zeeuw, Targeting of doxorubicin to the urinary bladder of the rat shows increased cytotoxicity in the bladder urine combined with an absence of renal toxicity, J. Drug Target. 10 (2002) 81–89.

[215] L. Guo, W. Ding, L.M. Zheng, The C(RgdyK)-conjugated Fe3O4 nanoparticles with high drug load for dual-targeting integrin alpha(v)beta3-expressing cancer cells, J. Nanosci. Nanotechnol. 14 (2014) 4858–4864.

[216] P. Huang, H. Song, W. Wang, Y. Sun, J. Zhou, X. Wang, J. Liu, J. Liu, D. Kong, A. Dong, Integrin-targeted zwitterionic polymeric nanoparticles with acid-induced disassembly property for enhanced drug accumulation and release in tumor, Biomacromolecules 15 (2014) 3128–3138, http://dx.doi.org/10.1021/bm500764p.

[217] C. Nazli, G.S. Demirer, Y. Yar, H.Y. Acar, S. Kizilel, Targeted delivery of doxorubicin into tumor cells via MMP-sensitive PEG hydrogel-coated magnetic iron oxide nanoparticles (MIONPs), Colloids Surf. B: Biointerfaces 122 (2014) 674–683, http://dx.doi.org/10.1016/j.colsurfb.2014.07.049.

[218] A. Gianella, A.J. Mieszawska, F.J. Hoeben, H.M. Janssen, P.A. Jarzyna, D.P. Cormode, K.D. Costa, S. Rao, O.C. Farokhzad, R. Langer, Z.A. Fayad, W.J. Mulder, Synthesis and in vitro evaluation of a multifunctional and surface-switchable nanoemulsion platform, Chem. Commun. (Camb.) 49 (2013) 9392–9394, http://dx.doi.org/10.1039/c3cc43618g.

[219] T. Jiang, E.S. Olson, Q.T. Nguyen, M. Roy, P.A. Jennings, R.Y. Tsien, Tumor imaging by means of proteolytic activation of cell-penetrating peptides, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17867–17872.
[220] J.L. Crisp, E.N. Savariar, H.L. Glasgow, L.G. Ellies, M.A. Whitney, R.Y. Tsien, Dual targeting of integrin αvβ3 and matrix metalloproteinase-2 for optical imaging of tumors and chemotherapeutic delivery, Mol. Cancer Ther. 13 (2014) 1514–1525, http://dx.doi.org/10.1158/1535-7163.MCT-13-1067.
[221] L. Buckel, E.N. Savariar, J.L. Crisp, K.A. Jones, A.M. Hicks, D.J. Scanderbeg, Q.T. Nguyen, J.K. Sicklick, A.M. Lowy, R.Y. Tsien, S.J. Advani, Tumor radiosensitization by monomethyl auristatin E: mechanism of action and targeted delivery, Cancer Res. 75 (2015) 1376–1387, http://dx.doi.org/10.1158/0008-5472.CAN-14-1931.
[222] P.D. Senter, E.L. Sievers, The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma, Nat. Biotechnol. 30 (2012) 631–637, http://dx.doi.org/10.1038/nbt.2289.
[223] L. Zhu, J. Xie, M. Swierczewska, F. Zhang, X. Lin, X. Fang, G. Niu, S. Lee, X. Chen, Dual-functional, receptor-targeted fluorogenic probe for in vivo imaging of extracellular protease expressions, Bioconjug. Chem. 22 (2011) 1001–1005, http://dx.doi.org/10.1021/bc200005w.
[224] E. Mebrahtu, A. Zheleznyak, M.A. Hur, R. Laforest, S.E. Lapi, Initial characterization of a dually radiolabeled peptide for simultaneous monitoring of protein targets and enzymatic activity, Nucl. Med. Biol. 40 (2013) 190–196, http://dx.doi.org/10.1016/j.nucmedbio.2012.10.003.
[225] C.Y. Quan, J.X. Chen, H.Y. Wang, C. Li, C. Chang, X.Z. Zhang, R.X. Zhuo, Core–shell nanosized assemblies mediated by the alpha–beta cyclodextrin dimer with a tumor-triggered targeting property, ACS Nano 4 (2010) 4211–4219, http://dx.doi.org/10.1021/nn100534q.
[226] C. Sun, H. Yang, Y. Yuan, X. Tian, L. Wang, Y. Guo, L. Xu, J. Lei, N. Gao, G.J. Anderson, X.J. Liang, C. Chen, Y. Zhao, G. Nie, Controlling assembly of paired gold clusters within apoferritin nanoreactor for in vivo kidney targeting and biomedical imaging, J. Am. Chem. Soc. 133 (2011) 8617–8624, http://dx.doi.org/10.1021/ja200746p.
[227] V.P. Torchilin, Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery, Nat. Rev. Drug Discov. 13 (2014) 813–827, http://dx.doi.org/10.1038/nrd4333.
[228] C. Casagrande, D. Arosio, C. Kusmic, L. Manzoni, L. Menichetti, A. L'Abbate, Molecular targeting of imaging and drug delivery probes in atherosclerosis, Ann. Rep. Med. Chem. 48 (2013) 105–118, http://dx.doi.org/10.1016/B978-0-12-417150-3.00008-9.
[229] A.J. Beer, H. Kessler, H.J. Wester, M. Schwaiger, PET imaging of integrin αVβ3 expression, Theranostics 1 (2011) 48–57.
[230] F. Xie, J. Lof, T. Matsunaga, R. Zutshi, T.R. Porter, Diagnostic ultrasound combined with glycoprotein IIb/IIIa-targeted microbubbles improves microvascular recovery after acute coronary thrombotic occlusions, Circulation 119 (2009) 1378–1385, http://dx.doi.org/10.1161/CIRCULATIONAHA.108.825067.
[231] S. Kaul, Sonothrombolysis: a universally applicable and better way to treat acute myocardial infarction and stroke? Who is going to fund the research? Circulation 119 (2009) 1358–1360, http://dx.doi.org/10.1161/CIRCULATIONAHA.108.846113.
[232] Y.Y. Bai, X. Gao, Y.C. Wang, X.G. Peng, D. Chang, S. Zheng, C. Li, S. Ju, Image-guided pro-angiogenic therapy in diabetic stroke mouse models using a multi-modal nanoprobe, Theranostics 4 (2014) (2014) 787–797, http://dx.doi.org/10.7150/thno.9525.eCollection.
[233] P.M. Peiris, R. Toy, E. Doolittle, J. Pansky, A. Abramowski, M. Tam, P. Vicente, E. Tran, E. Hayden, A. Camann, A. Mayer, B.O. Erokwu, Z. Berman, D. Wilson, H. Baskaran, C.A. Flask, R.A. Keri, E. Karathanasis, Imaging metastasis using an integrin-targeting chain-shaped nanoparticle, ACS Nano 6 (2012) 8783–8795, http://dx.doi.org/10.1021/nn303833p.
[234] P.M. Peiris, R. Toy, A. Abramowski, P. Vicente, S. Tucci, L. Bauer, A. Mayer, M. Tam, E. Doolittle, J. Pansky, E. Tran, D. Lin, W.P. Schiemann, K.B. Ghaghada, M.A. Griswold, E. Karathanasis, Treatment of cancer micrometastasis using a multicomponent chain-like nanoparticle, J. Control. Release 173 (2014) 51–58, http://dx.doi.org/10.1016/j.jconrel.2013.10.031.
[235] S.Y. Lee, M. Ferrari, P. Decuzzi, Design of bio-mimetic particles with enhanced vascular interaction, J. Biomech. 42 (2009) 1885–1890, http://dx.doi.org/10.1016/j.jbiomech.2009.05.012.
[236] P.M. Peiris, E. Schmidt, M. Calabrese, E. Karathanasis, Assembly of linear nanochains from iron oxide nanospheres with asymmetric surface chemistry, PLoS One 6 (2011) e15927, http://dx.doi.org/10.1371/journal.pone.0015927.
[237] M.S. Kim, D.W. Lee, K. Park, S.J. Park, E.J. Choi, E.S. Park, H.R. Kim, Temperature-triggered tumor-specific delivery of anticancer agents by cRGD-conjugated thermosensitive liposomes, Colloids Surf. B: Biointerfaces 116 (2014) 17–25, http://dx.doi.org/10.1016/j.colsurfb.2013.12.045.
[238] M.P. Melancon, R.J. Stafford, C. Li, Challenges to effective cancer nanotheranostics, J. Control. Release 164 (2012) 177–182, http://dx.doi.org/10.1016/j.jconrel.2012.07.045.
[239] W. Lu, M.P. Melancon, C. Xiong, Q. Huang, A. Elliott, S. Song, R. Zhang, L.G. Flores II, J.G. Gelovani, L.V. Wang, G. Ku, R.J. Stafford, C. Li, Effects of photoacoustic imaging and photothermal ablation therapy mediated by targeted hollow gold nanospheres in an orthotopic mouse xenograft model of glioma, Cancer Res. 71 (2011) 6116–6121, http://dx.doi.org/10.1158/0008-5472.CAN-10-4557.
[240] M.P. Melancon, M. Zhou, C. Li, Cancer theranostics with near-infrared light-activatable multimodal nanoparticles, Acc. Chem. Res. 44 (2011) 947–956, http://dx.doi.org/10.1021/ar200022e.
[241] R. Zhang, D. Pan, X. Cai, X. Yang, A. Senpan, J.S. Allen, G.M. Lanza, L.V. Wang, $\alpha_v\beta_3$-targeted copper nanoparticles incorporating an Sn 2 lipase-labile fumagillin prodrug for photoacoustic neovascular imaging and treatment, Theranostics 5 (2015) 124–133, http://dx.doi.org/10.7150/thno.10014 (eCollection 2015).
[242] S. Sirsi, M. Borden, Microbubble compositions, properties and biomedical applications, Bubble Sci. Eng. Technol. 1 (2009) 3–17.
[243] J.R. Lindner, Molecular imaging of myocardial and vascular disorders with ultrasound, JACC Cardiovasc. Imaging 3 (2010) 204–211, http://dx.doi.org/10.1016/j.jcmg.2009.09.021.
[244] E.F. Plow, M. Das, Rising like the phoenix? Arterioscler. Thromb. Vasc. Biol. 34 (2014) 2182–2183, http://dx.doi.org/10.1161/ATVBAHA.114.304486.
[245] D.B. Ellegala, H. Leong-Poi, J.E. Carpenter, A.L. Klibanov, S. Kaul, M.E. Shaffrey, J. Sklenar, J.R. Lindner, Imaging tumor angiogenesis with contrast ultrasound and microbubbles targeted to alpha(v)beta3, Circulation 108 (2003) 336–341.
[246] M.S. Tartis, J. McCallan, A.F. Lum, R. LaBell, S.M. Stieger, T.O. Matsunaga, K.W. Ferrara, Therapeutic effects of paclitaxel-containing ultrasound contrast agents, Ultrasound Med. Biol. 32 (2006) 1771–1780.
[247] F. Kiessling, S. Fokong, P. Koczera, W. Lederle, T. Lammers, Ultrasound microbubbles for molecular diagnosis, therapy, and theranostics, J. Nucl. Med. 53 (2012) 345–348, http://dx.doi.org/10.2967/jnumed.111.099754.
[248] Y.H. Li, Q.S. Shi, J. Du, L.F. Jin, L.F. Du, P.F. Liu, Y.R. Duan, Targeted delivery of biodegradable nanoparticles with ultrasound-targeted microbubble destruction-mediated hVEGF-siRNA transfection in human PC-3 cells in vitro, Int. J. Mol. Med. 31 (2013) 163–171, http://dx.doi.org/10.3892/ijmm.2012.1175.
[249] C. Scaringi, G. Minniti, P. Caporello, R.M. Enrici, Integrin inhibitor cilengitide for the treatment of glioblastoma: a brief overview of current clinical results, Anticancer Res. 32 (2012) 4213–4223.
[250] M. Preusser, D. Capper, A. Ilhan-Mutlu, A.S. Berghoff, P. Birner, R. Bartsch, C. Marosi, C. Zielinski, M.P. Mehta, F. Winkler, W. Wick, A. von Deimling, Brain metastases: pathobiology and emerging targeted therapies, Acta Neuropathol. 123 (2012) 205–222, http://dx.doi.org/10.1007/s00401-011-0933-9.
[251] D. Allhenn, M.A. Boushehri, A. Lamprecht, Drug delivery strategies for the treatment of malignant gliomas, Int. J. Pharm. 436 (2012) 299–310, http://dx.doi.org/10.1016/j.ijpharm.2012.06.025.
[252] U. Agrawal, G. Chashoo, P.R. Sharma, A. Kumar, A.K. Saxena, S.P. Vyas, Tailored polymer–lipid hybrid nanoparticles for the delivery of drug conjugate: dual strategy for brain targeting, Colloids Surf. B: Biointerfaces 126 (2015) 414–425, http://dx.doi.org/10.1016/j.colsurfb.2014.12.045.
[253] P. Zhang, L. Hu, Q. Yin, L. Feng, Y. Li, Transferrin-modified c[RGDfK]-paclitaxel loaded hybrid micelle for sequential blood–brain barrier penetration and glioma targeting therapy, Mol. Pharm. 9 (2012) 1590–1598, http://dx.doi.org/10.1021/mp200600t.
[254] 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, http://dx.doi.org/10.1021/nn402662d.
[255] H. Ding, S. Inoue, A.V. Ljubimov, R. Patil, J. Portilla-Arias, J. Hu, B. Konda, K.A. Wawrowsky, M. Fujita, N. Karabalin, T. Sasaki, K.L. Black, E. Holler, J.Y. Ljubimova, Inhibition of brain tumor growth by intravenous poly (β-L-malic acid) nanobioconjugate with pH-dependent drug release [corrected], Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 18143–18148, http://dx.doi.org/10.1073/pnas.1003919107 (Erratum in: Proc Natl Acad Sci U S A. 107 (2010) 19603).
[256] M. Fujita, B.S. Lee, N.M. Khazenzon, M.L. Penichet, K.A. Wawrowsky, R. Patil, H. Ding, E. Holler, K.L. Black, J.Y. Ljubimova, Brain tumor tandem targeting using a combination of monoclonal antibodies attached to biopoly(beta-L-malic acid), J. Control. Release 122 (2007) 356–363.
[257] P.M. Peiris, A. Abramowski, J. Mcginnity, E. Doolittle, R. Toy, R. Gopalakrishnan, S. Shah, L. Bauer, K.B. Ghaghada, C. Hoimes, S.M. Brady-Kalnay, J.P. Basilion, M.A. Griswold, E. Karathanasis, Treatment of invasive brain tumors using a chain-like nanoparticle, Cancer Res. 75 (2015) 1356–1365, http://dx.doi.org/10.1158/0008-5472.CAN-14-1540.
[258] L. Agemy, V.R. Kotamraju, D. Friedmann-Morvinski, S. Sharma, K.N. Sugahara, E. Ruoslahti, Proapoptotic peptide-mediated cancer therapy targeted to cell surface p32, Mol. Ther. 21 (2013) 2195–2204, http://dx.doi.org/10.1038/mt.2013.191.
[259] G.M. D'Abaco, A.H. Kaye, Integrins: molecular determinants of glioma invasion, J. Clin. Neurosci. 14 (2007) 1041–1048.
[260] W.S. Carbonell, M. DeLay, A. Jahangiri, C.C. Park, M.K. Aghi, β1 integrin targeting potentiates antiangiogenic therapy and inhibits the growth of bevacizumab-resistant glioblastoma, Cancer Res. 73 (2013) 3145–3154, http://dx.doi.org/10.1158/0008-5472.CAN-13-0011.
[261] T. Wang, J.R. Upponi, V.P. Torchilin, Design of multifunctional non-viral gene vectors to overcome physiological barriers: dilemmas and strategies, Int. J. Pharm. 427 (2012) 3–20, http://dx.doi.org/10.1016/j.ijpharm.2011.07.013.
[262] J.D. Hood, M. Bednarski, R. Frausto, S. Guccione, R.A. Reisfeld, R. Xiang, D.A. Cheresh, Tumor regression by targeted gene delivery to the neovasculature, Science 296 (2002) 2404–2407.
[263] J. Park, K. Singha, S. Son, J. Kim, R. Namgung, C.O. Yun, W.J. Kim, A review of RGD-functionalized nonviral gene delivery vectors for cancer therapy, Cancer Gene Ther. 19 (2012) 741–748, http://dx.doi.org/10.1038/cgt.2012.64.
[264] R. Kole, A.R. Krainer, S. Altman, RNA therapeutics: beyond RNA interference and antisense oligonucleotides, Nat. Rev. Drug Discov. 11 (2012) 125–140, http://dx.doi.org/10.1038/nrd3625.
[265] R. Juliano, J. Bauman, H. Kang, X. Ming, Biological barriers to therapy with antisense and siRNA oligonucleotides, Mol. Pharm. 6 (2009) 686–695, http://dx.doi.org/10.1021/mp900093r.
[266] M.R. Alam, X. Ming, M. Fisher, J.G. Lackey, K.G. Rajeev, M. Manoharan, R.L. Juliano, Multivalent cyclic RGD conjugates for targeted delivery of small interfering RNA, Bioconjug. Chem. 22 (2011) 1673–1681, http://dx.doi.org/10.1021/bc200235q.

[267] X. Liu, W. Wang, D. Samarsky, L. Liu, Q. Xu, W. Zhang, G. Zhu, P. Wu, X. Zuo, H. Deng, J. Zhang, Z. Wu, X. Chen, L. Zhao, Z. Qiu, Z. Zhang, Q. Zeng, W. Yang, B. Zhang, A. Ji, Tumor-targeted in vivo gene silencing via systemic delivery of cRGD-conjugated siRNA, Nucleic Acids Res. 42 (2014) 11805–11817, http://dx.doi.org/10.1093/nar/gku831.
[268] C. Dufès, I.F. Uchegbu, A.G. Schätzlein, Dendrimers in gene delivery, Adv. Drug Deliv. Rev. 57 (2005) 2177–2202.
[269] G. Li, Z. Hu, H. Yin, Y. Zhang, X. Huang, S. Wang, W. Li, A novel dendritic nanocarrier of polyamidoamine–polyethylene glycol–cyclic RGD for "smart" small interfering RNA delivery and in vitro antitumor effects by human ether-à-go-go-related gene silencing in anaplastic thyroid carcinoma cells, Int. J. Nanomedicine 8 (2013) 1293–1306, http://dx.doi.org/10.2147/IJN.S41555.
[270] L.A. Pardo, C. Contreras-Jurado, M. Zientkowska, F. Alves, W. Stühmer, Role of voltage-gated potassium channels in cancer, J. Membr. Biol. 205 (2005) 115–124.
[271] X. Liu, C. Liu, C. Chen, M. Bentobji, F.A. Cheillan, J.T. Piana, F. Qu, P. Rocchi, L. Peng, Targeted delivery of Dicer-substrate siRNAs using a dual targeting peptide decorated dendrimer delivery system, Nanomedicine 10 (2014) 1627–1636, http://dx.doi.org/10.1016/j.nano.2014.05.008.
[272] M. Ou, X.L. Wang, R. Xu, C.W. Chang, D.A. Bull, S.W. Kim, Novel biodegradable poly (disulfide amine)s for gene delivery with high efficiency and low cytotoxicity, Bioconjug. Chem. 19 (2008) 626–633, http://dx.doi.org/10.1021/bc700397x.
[273] H.A. Kim, K. Nam, S.W. Kim, Tumor targeting RGD conjugated bio-reducible polymer for VEGF siRNA expressing plasmid delivery, Biomaterials 35 (2014) 7543–7552, http://dx.doi.org/10.1016/j.biomaterials.2014.05.021.
[274] Q. Leng, P. Scaria, P. Lu, M.C. Woodle, A.J. Mixson, Systemic delivery of HK Raf-1 siRNA polyplexes inhibits MDA-MB-435 xenografts, Cancer Gene Ther. 15 (2008) 485–495, http://dx.doi.org/10.1038/cgt.2008.29.
[275] S.T. Chou, Q. Leng, P. Scaria, M. Woodle, A.J. Mixson, Selective modification of HK peptides enhances siRNA silencing of tumor targets in vivo, Cancer Gene Ther. 18 (10) (2011 Oct) 707–716, http://dx.doi.org/10.1038/cgt.2011.40.
[276] S.T. Chou, Q. Leng, P. Scaria, J.D. Kahn, L.J. Tricoli, M. Woodle, A.J. Mixson, Surface-modified HK:siRNA nanoplexes with enhanced pharmacokinetics and tumor growth inhibition, Biomacromolecules 14 (2013) 752–760, http://dx.doi.org/10.1021/bm3018356.
[277] H. Ragelle, G. Vandermeulen, V. Préat, Chitosan-based siRNA delivery systems, J. Control. Release 172 (1) (2013 Nov 28) 207–218, http://dx.doi.org/10.1016/j.jconrel.2013.08.005.
[278] P. Ghosh, G. Han, M. De, C.K. Kim, V.M. Rotello, Gold nanoparticles in delivery applications, Adv. Drug Deliv. Rev. 60 (2008) 1307–1315, http://dx.doi.org/10.1016/j.addr.2008.03.016.
[279] Y. Ding, Z. Jiang, K. Saha, C.S. Kim, S.T. Kim, R.F. Landis, V.M. Rotello, Gold nanoparticles for nucleic acid delivery, Mol. Ther. 22 (2014) 1075–1083, http://dx.doi.org/10.1038/mt.2014.30.
[280] J. Conde, A. Ambrosone, V. Sanz, Y. Hernandez, V. Marchesano, F. Tian, H. Child, C.C. Berry, M.R. Ibarra, P.V. Baptista, C. Tortiglione, J.M. de la Fuente, Design of multifunctional gold nanoparticles for in vitro and in vivo gene silencing, ACS Nano 6 (2012) 8316–8324.
[281] J. Conde, F. Tian, Y. Hernandez, C. Bao, D. Cui, K.P. Janssen, M.R. Ibarra, P.V. Baptista, T. Stoeger, J.M. de la Fuente, In vivo tumor targeting via nanoparticle-mediated therapeutic siRNA coupled to inflammatory response in lung cancer mouse models, Biomaterials 34 (2013) 7744–7753, http://dx.doi.org/10.1016/j.biomaterials.2013.06.041.
[282] J.H. Lee, K. Lee, S.H. Moon, Y. Lee, T.G. Park, J. Cheon, All-in-one target-cell specific magnetic nanoparticles for simultaneous molecular imaging and siRNA delivery, Angew. Chem. Int. Ed. Engl. 48 (2009) 4174–4179, http://dx.doi.org/10.1002/anie.200805998.
[283] J. Qian, X. Gao, Triblock copolymer-encapsulated nanoparticles with outstanding colloidal stability for siRNA delivery, ACS Appl. Mater. Interfaces 5 (2013) 2845–2852, http://dx.doi.org/10.1021/am3021813.
[284] X.Q. Liu, W.J. Song, T.M. Sun, P.Z. Zhang, J. Wang, Targeted delivery of antisense inhibitor of miRNA for antiangiogenesis therapy using cRGD-functionalized nanoparticles, Mol. Pharm. 8 (2011) 250–259, http://dx.doi.org/10.1021/mp100315q.
[285] M.Z. Zhang, C. Li, B.Y. Fang, M.H. Yao, Q.Q. Ren, L. Zhang, Y.D. Zhao, High transfection efficiency of quantum dot antisense oligonucleotide nanoparticles in cancer cells through dual-receptor synergistic targeting, Nanotechnology 25 (2014) 255102, http://dx.doi.org/10.1088/0957-4484/25/25/255102.