Expert Opinion

Integrin targeted drug and gene delivery

Zhe Wang, Wai-Keung Chui & Paul C Ho†
National University of Singapore, Department of Pharmacy, 18 Science Drive 4, Singapore 117543, Singapore

Importance of the field: Recently, there has been substantial progress in the development of integrin targeted pharmaceuticals and drug delivery systems. Integrin is an important member in the cell adhesion molecule family, which is involved in regulation of complex biological conditions, from keeping normal physiological activities to causing cellular dysfunction in diseased cells. Hence, it is timely to summarize the recent developments in integrin targeted drug and gene delivery systems to understand better their advantages and limitations.

Areas covered in this review: In this review, advances in the discovery and clinical trials of these integrin antagonists against different integrin subunits are summarized and discussed. Besides using integrin inhibitor as a single therapeutic agent, integrin antagonists that were conjugated to cytotoxic drugs by synthetic chemistry or coupled to biomacromolecules by either DNA recombination technology or fusion protein technology for integrin targeted therapy have been explored. Furthermore, nanoparticles with integrin targeting ligands for both drug and gene delivery, typically for antiangiogenesis and anticancer therapy, are highlighted and evaluated.

What the reader will gain: This review sheds light on the future development of integrin targeted drug and/or gene delivery systems.

Take home message: Although thus far there are still limitations, integrin targeted delivery systems have already shown their potential as important pharmaceuticals in the near future.

Keywords: angiogenesis, antagonist, conjugation, drug delivery, integrin targeting

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1. Introduction

In past decades, much effort has been given to the discovery of cell adhesion molecules (CAMs), and their distinctive functions in cell morphology, mitosis, maintenance of cellular polarity, pathological regulations and mutation in disease progression have also been investigated extensively [1,2]. Cell adhesion molecules play key roles in the human body to keep its integrity by regulating cell–cell and/or cell–extracellular matrix (ECM) connections. CAMs have been shown to be important in various diseases, such as cancer [3], thrombosis [4,5], arthritis [6,7] and diabetes [8].

Generally, CAMs can be divided into four classes: integrins, cadherins, selectins and immunoglobulin superfamily. Among them, ingetrins have drawn much attention in both clinical and academic research because of their vital roles in mediating various diseases. Integrins are cellular surface glycoprotein receptors consisting of a heterodimer of α- and β-subunits that are mutually non-covalently associated. In mammals, integrins have extensive distributions throughout the whole body (**Table 1**), and there are 18 α- and 8 β-subunits assembling 24 functionally different heterodimers [9,10]. Each individual integrin subunit has a large extracellular domain, a single membrane-spanning domain and a short non-catalytic cytomplasmic tail [11]. The assembled integrin heterodimer can bind to a unique

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Article highlights.

- An overview on cell adhesion molecules.
- The development of various integrin antagonists, their application and limitations.
- Their objectives and accomplishment so far.
- The rationales and examples.
- Exploration for cancer therapy and treatment of other diseases.
- The integrin drug and gene delivery systems have great potential for treatment of diseases in human healthcare.
- It is always a challenge to develop safer, less complicated structures and more effective drug and gene delivery systems.

This box summarises key points contained in the article.

set of ligands in the ECM, soluble ligands and ligands on other cellular membrane surface as cellular cross-talking [12]. On ligand binding, integrins cluster into focal contracts containing many different actin-associated proteins, such as α-actin, vinculin, tensin and paxillin, which link the integrin to the cytoskeleton [11,13]. The binding association can initiate intracellular signaling events, and also activate kinase that phosphorylates cytoskeletal proteins [14]. In turn, the reassembling of actin filaments into stress fibers stimulates integrin clustering and enhances the affinity with ECM proteins [15].

As integrins play diverse roles in several distinct physiological and disease processes, various integrin targeting methods have been explored extensively to mediate physical regulation or deliver medicines to specific cells for treatment. In this review, the most recent advancement of integrin targeted drug and gene delivery are focused on. In particular, integrin-based drug and gene delivery for anticancer and antiangiogenesis therapy are discussed in more detail.

2. Integrin antagonists

The complexity, structural variety and functional diversity of integrins make this superfamily of cell adhesion molecules play a vital role in the context of many biological activities, including innate or antigen-specific immunogenicity, inflammation, hemostasis, wound healing, tissue morphogenesis, and regulation of cell growth, migration and differentiation [16,17]. On the other hand, dysregulation of integrins results in pathological diseases from autoimmunogenesis to thrombotic vascular disorder to angiogenesis of cancer and its metastasis [18,19]. In addition, because angiogenetic endothelial cells of solid tumors overexpress several integrins, such as $\alpha_V\beta_3$, whereas pre-existing resting normal blood vessel can hardly be detected for those integrins, it is of great interest to use the integrin antagonists to inhibit angiogenesis of solid tumors in cancer therapy. Thus, it is essential to discover and develop integrin antagonists for clinical applications. So far, many integrin antagonists have been discovered or developed, including monoclonal antibodies, peptides, non-peptide small molecules, and so on, and some of these antagonists are or will be available on the market.

2.1 Antibodies

c7E3 (abciximab, ReoPro®, Centocor, Inc., Philidelphia, PA, USA & Eli Lilly, Indiana) is a mouse/human chimera derived from a mouse anti-$\alpha_{IIb}\beta_3$ monoclonal antibody. This antibody remains the variable region of mouse anti-$\alpha_{IIb}\beta_3$ monoclonal antibody, 7E3, but ligated to the constant domain of human immunoglobulin (IgG). It has been reported to show inhibition of platelet aggregation [20] by targeting GPIIb–IIIa ($\alpha_{IIb}\beta_3$) on platelets. Not only can the c7E3 bind to $\alpha_{IIb}\beta_3$, but also it binds to $\alpha_V\beta_3$ integrin at equivalent affinity [21], and tends to redistribute between these two receptors [22]. As $\alpha_V\beta_3$ integrin is important in mediating pathological neovascularization, cancer angiogenesis and metastasis [23], and c7E3 has high affinity to $\alpha_V\beta_3$, it was reported that c7E3 inhibited $\alpha_V\beta_3$-mediated human umbilical vein endothelial (HUVEC) and melanoma cell adhesion, migration and invasion, and basic fibroblast growth factor (bFGF) stimulated proliferation of HUVECs in the *in vitro* angiogenesis study [21]. In a human tumor xenograft study, c7E3 showed potency in inhibiting tumor angiogenesis and growth [24], and even completely blocked tumor formation and growth of human melanoma tumors growing in nude rats [21]. Owing to the success of the preclinical experiment results, c7E3 has passed large-scale clinical trials on 2099 patients undergoing coronary intervention, which aimed to investigate the protective effect to those patients [25]. In another clinical trial, c7E3 was used against GPIIb–IIIa integrin to reduce clinical restenosis, and a 26% reduction effect was still seen even after 6 months [26]. However, there has not yet been any clinical report on the use of c7E3 for treatment of cancers.

LM609 is a mouse anti-human monoclonal antibody raised against $\alpha_V\beta_3$ integrin [27]. The specific and effective blocking of bFGF and tumor necrosis factor-α (TNF-α) of LM609 was its primary antiangiogenesis mechanism [28]. After intravenous injection, LM609 effectively reduced tumor angiogenesis and growth [23,29]; meanwhile, it had little, if any, impact on pre-existing resting vessels [30]. Rader and co-workers described the *in vitro* affinity maturation of humanized LM609 by using a phage display strategy for the sequential and parallel optimization of three complementarity determining regions of the antibody [31]. The humanized version of LM609 showed improved affinity to $\alpha_V\beta_3$ integrin and, importantly, demonstrated selective targeting to human Kaposi's sarcoma and significantly inhibited tumor growth in an animal model [31]. Nevertheless, clinical trials have found its limitation for metastatic cancer treatment [32], probably owing to the single integrin ($\alpha_V\beta_3$) targeting characteristic of LM609. As malignant metastatic cancers are believed to involve more than one integrin pathway, other integrins, such as $\alpha_V\beta_5$ or $\alpha_5\beta_1$, and so on, are also able to mediate tumor angiogenesis and

Table 1. List of the integrin subunit combinations, their distribution and RGD recognition.

Integrin subunit		Integrin distribution sites	RGD recognition ±
$\alpha_1\beta_1$		Laminins, collagens	-
$\alpha_2\beta_1$		Laminins, collagens, chodroadherin	-
$\alpha_3\beta_1$		Laminins, fibronectin, thrombospondin, collagen, epiligrin, entactin	+
$\alpha_4\beta_1$		VCAM, fibronectin	-
$\alpha_4\beta_7$		VCAM, MAdCAM, fibronectin	NA
$\alpha_5\beta_1$		Fibronectin, fibrinogen	+
$\alpha_6\beta_1$		Merosin, kalinin, laminins	-
$\alpha_6\beta_4$		Laminins	NA
$\alpha_7\beta_1$		Laminins	-
$\alpha_8\beta_1$		Fibronectin, vitronectin, osteopontin, nephronectin, tenascin-C	-
$\alpha_9\beta_1$		Tenascin, angiostatin, tenascin-C, osteopontin, VCAM-1, tTG	-
$\alpha_{10}\beta_1$		Collagens	NA
$\alpha_{11}\beta_{IIIa}$		Fibronectin, fibrogen, fibroblast, von Willebrand factor, vitronectin	+
$\alpha_D\beta_2$		VCAM, ICAM	NA
$\alpha_E\beta_7$		E-cadherin	NA
$\alpha_L\beta_2$		ICAM-1,2,3	NA
$\alpha_M\beta_2$		Fibrinogen, ICAMs, denatured ovalbumin	-
$\alpha_X\beta_2$		Fibrinogen	-
$\alpha_{IIb}\beta_3$		Collagens, fibronectin, vitronectin, fibrinogen, von Willebrand factor, thrombospondin	+
α_v	β_1	Vitronectin, fibronectin	NA
	β_3	Fibronectin, vitronectin, fibrinogen, von Willebrand factor, thrombospondin, FGF2, metalloproteinase MMP-2, ADAM proteins	+
	β_5	Vitronectin	+
	β_6	Tenascin, fibronectin	+
	β_7	Fibronectin, collagens, laminins	NA
	β_8	Vitronectin, fibronectin	NA
	β_N	Fibronectin, collagens	NA

ADAM: A disintegrin-like and metalloproteinase; FGF2: Fibroblast growth factor 2; ICAM: Intercellular cell adhesion molecule; MAdCAM: Mucosal addressin cell adhesion molecule; NA: Not available; tTG: Tissue-type transglutaminase; VCAM: Vascular cell adhesion molecule.

growth [28]. Therefore, a combination of different integrin receptor pathways is believed to be more effective at inhibiting malignant cancer growth and metastasis [33].

There are some humanized monoclonal antibodies available for the combined targeting of more integrin receptors. Besides the as-mentioned c7E3 binding equally to both $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$, CNTO 95 was reported to have affinity to multiple α_V integrins, with especially high affinity to $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins [34,35]. The Phase I clinical trial of CNTO 95 showed penetration of the tumor and localization to tumor cells in association with reduced bcl-2 expression, and a lesion in 1 patient (10.0 mg/kg) with stable ovarian carcinosarcoma was no longer detectable by day 49 post-injection [36]. Besides being used as a single agent for cancer therapy, CNTO 95 was also reported to enhance synergistically the efficacy of radiotherapy. The combined therapy of CNTO 95 and fractionated radiation significantly inhibited tumor growth and produced the longer tumor growth delay time [37]. Similarly, another monoclonal antibody, 17E6, was raised not only against $\alpha_V\beta_3$, but also to $\alpha_V\beta_5$ and $\alpha_V\beta_1$ [38,39], and it caused dramatic inhibition of many malignant cancers, all of which over-expressed α_V integrins [39]. In summary, antibodies with multiple integrin binding affinity should be promising agents for integrin targeted cancer therapy for better clinical results.

2.2 Peptides

Besides antibodies, synthetic peptides that mimic the structure of natural integrin binding ligands are another important integrin antagonist group. Much attention has been paid to Arg-Gly-Asp (RGD) mimic peptides. As early as 1986, Ruoslahti and Pierschbacher discovered that the RGD sequence found in fibronectin was responsible for cell adhesion to fibronectin, and a small RGD peptide derived from fibronection was able to inhibit cell adhesion to fibronectin [40]. Besides fibronectin, other ECM glycoproteins also contain an

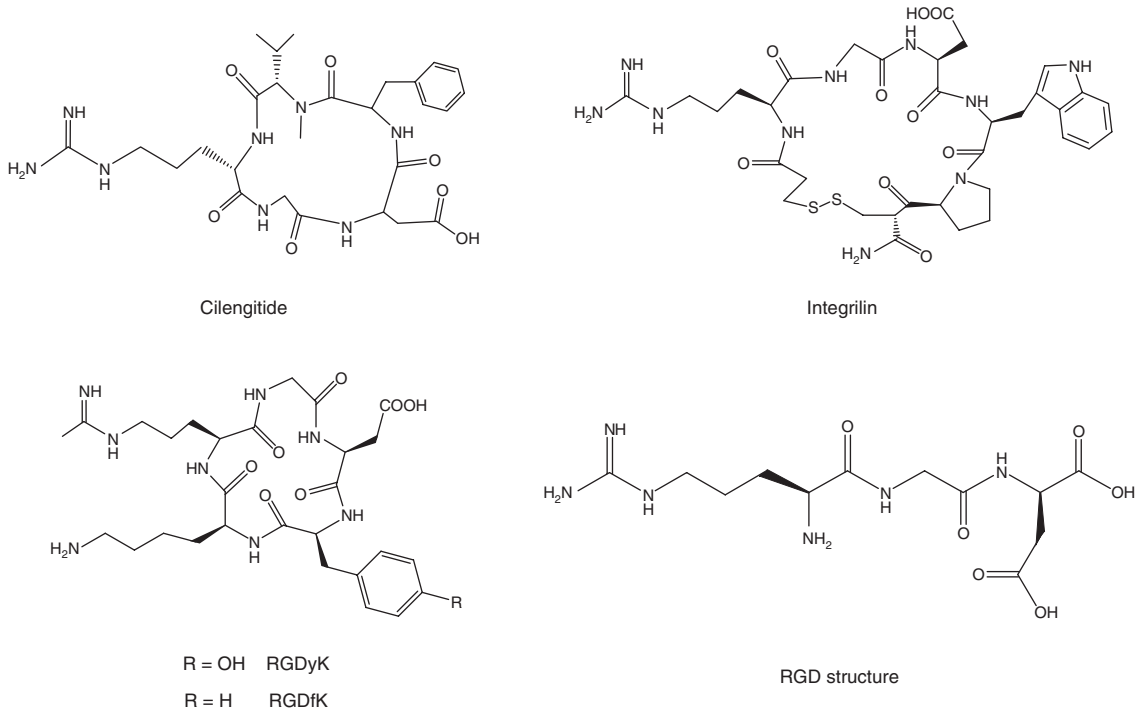

Figure 1. Representative peptides of integrin antagonists.

RGD motif in their structures, including fibrinogen, von Willebrand factor, vitronectin, osteonectin, thrombospondin, and proteolysed collagen and laminin [11]. In the literature, various integrins have been reported to recognize the RGD sequence (**Table 1**).

The binding selectivity of RGD triple-peptide to specific integrin depends much on the conformation of the RGD sequence and its flanking residues [41,42]. A single RGD triple-peptide has low bioavailability as pharmaceutics mainly because of the short half-life in the body. By conformation restriction of the RGD sequence and chemical modification, many researchers have developed a variety of cyclic RGD-containing peptides not only with diverse secondary structures of RGD peptides, but also with enhanced binding affinity and bioavailability [12,43]. In chemistry, the formation of cyclic RGD-containing peptides can be accomplished by forming either a peptide bond (i.e., cRGDfK, cRGDyK, Cilengitide) or a disulfide bond with the peptide sequence (i.e., RGDC4, Integrilin®, Schering-Plough, NJ, USA) (**Figure 1**).

Among many available RGD mimic peptides, Cilengitide (c[-RGDf(NMe)V-], EMD 121974, Merck KGaA) (**Figure 1**), a head-to-tail cyclic peptide containing the RGD triad wedged into a small constrained sequence flanked by D-phenylalanine and *N*-methylated valine residue [43], has emerged as a promising moiety. It can bind to both integrin $\alpha_V\beta_3$ (IC_{50} = 2.3 nM) and $\alpha_V\beta_5$ (IC_{50} = 37 nM) with high affinity [40], respectively. Cilengitide has been evaluated in several Phase I and Phase II trials [44-48]. In one of the Phase I clinical trials, a cohort of 51 patients suffering from recurrent malignant glioma was involved in the study. Cilengitide was reported to be well tolerated at a dose of 2400 mg/m^2, and 2 patients demonstrated complete response, 3 patients had partial response, and 4 patients had stable disease [48]. In another Phase I study, which focused on the children with refractory brain tumor, one patient with glioblastoma multiforme demonstrated complete response, and two patients had stable disease (SD). An extra patient had SD for > 5 months [44]. The promising results from this study led to the continuing Phase II study by the same research group. Other Phase II studies of Cilengitide on prostate cancer [46] and pancreatic cancer [47] have also taken place.

Similar to Cilengitide, a new antiangiogenesis peptide, ATN-161, has also drawn much attention recently. Similar to the origin of RGD-based peptides, ATN-161 is also derived from fibronectin, by replacing an arginine residue of the primary sequence with cysteine moiety, whereas it is a non-RGD-based peptide with five amino acid peptide binding to integrins $\alpha_V\beta_3$ and $\alpha_5\beta_1$ [49]. It has been demonstrated to inhibit tumor growth, angiogenesis and metastasis in multiple animal models [50-52]. Recent Phase I trial on patients with advanced solid tumor showed prolonged stale disease with ATN-161 [53].

Another important integrin antagonist peptide drug – Integrilin – is now on the market. It is an antithrombic drug, and functions by selectively binding to GPIIb-IIIa

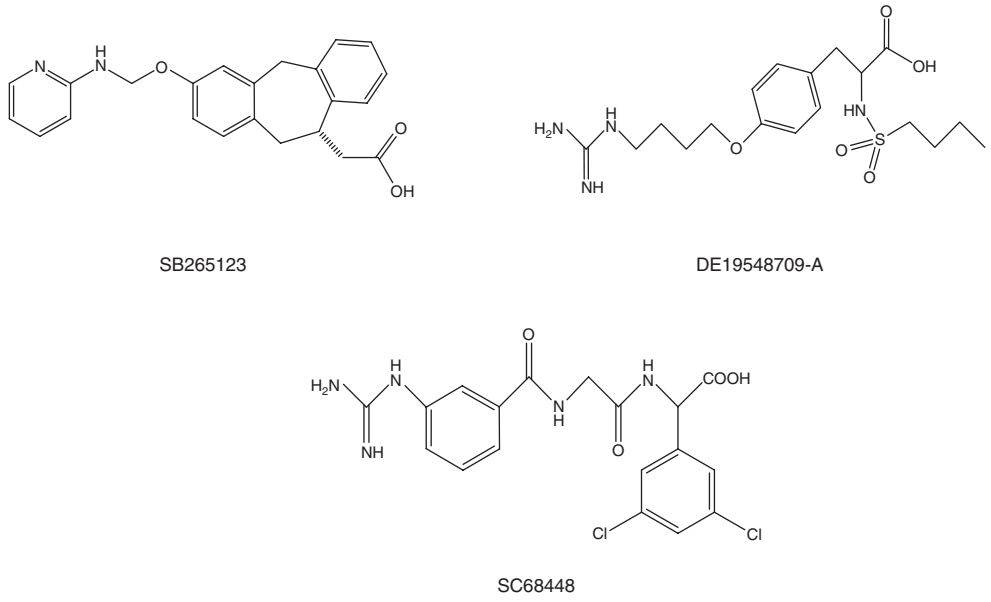

Figure 2. Selective examples of small molecules of integrin $\alpha_V\beta_3$ antagonists.

receptors (integrin $\alpha_{IIb}\beta_3$) on platelet cell surface [54]. During thrombosis, fibrinogen binds to gpIIb–IIIa receptors to generate platelet aggregation. Integrilin is able to inhibit competitively the fibrinogen binding with gpIIb–IIIa receptors, thus preventing the platelet aggregation effectively [55].

Leukocyte function-associated antigen-1 (LFA-1) integrin is another target leading to the discovery of a series of peptides [15]. These peptides were found to block mixed lymphocyte reactions and heterotypic T-cell adhesion to intestinal mucosa epithelia cell monolayers, and pancreatic islet microvascular endothelial cells [56,57]. Cyclic peptide cLABL derived from LFA-1 was reported to bind to intercellular adhesion molecule-1 (ICAM-1), and internalized into cytoplasm domain by means of ICAM-1. Therefore, it has been considered as a potential targeting ligand for drug delivery into ICAM-1 overexpressing cells [58].

Disintegrins identified from viper snake venoms is a family of low-molecular-mass RGD proteins. Generally, disintegrins can be divided into monomeric and dimeric groups [59]. The former group consists of three subgroups different in their peptide length with several disulfide bonds [60]; the latter group contains homodimeric and heterodimeric disintegrins [61]. In 1987, Huang and co-workers reported the first disintegrin – trigramin – which inhibits fibrinogen interaction with platelet receptor gpIIb/IIIa [62]. After that, many disintegrins were discovered, and some of them are promising for clinical trials [59].

2.3 Small molecules: peptidomimetics and other integrin antagonists

From monoclonal antibodies to peptides to disintegrins, all are biological products as integrin antagonists. Although there have been clinical trials, and some are even already on the market, these biological moieties are not without limitations, such as high price, difficult to scale up, low bioavailability, enzyme susceptibility, and so on. Fortunately, the chemically synthesized products that mimic the functions and structures of the biological integrin antagonists hold great potential for developing into suitable therapeutic agents. Peptidomimetic belongs to the chemically synthesized integrin antagonist family. It is synthesized by non-peptide elements that mimic the natural peptide's biological structures and activity. The most significant advantage of peptidomimetic lies in its ability not to be degraded enzymatically owing to its non-peptide characteristic. Thus, it potentially has higher oral bioavailability. Moreover, peptidomimetic can be synthesized and scaled-up by combinatorial chemistry technology [63]. These two advantages make peptidomimetics attractive in the pharmaceutical industry. Some selected non-peptide $\alpha_V\beta_3$ integrin antagonists are listed in **Figure 2**.

3. Conjugation of drug with ligand for integrin targeting

As integrins are transmemebrane glycoproteins and can be internalized by cells on activation with anchoring ligands, this characteristic can be used to facilitate delivery of medicines into cancerous cells and leukocytes. In addition, integrins are overexpressed on angiogenetic endothelial cells and neovascular cells, whereas they are absent in pre-existing endothelial cells and normal organs; and some tumor cells also express integrins on their cellular membrane surface. All of these characteristics make integrins good targeting ligands for both tumor and antiangiogenesis targeting therapy. In recent

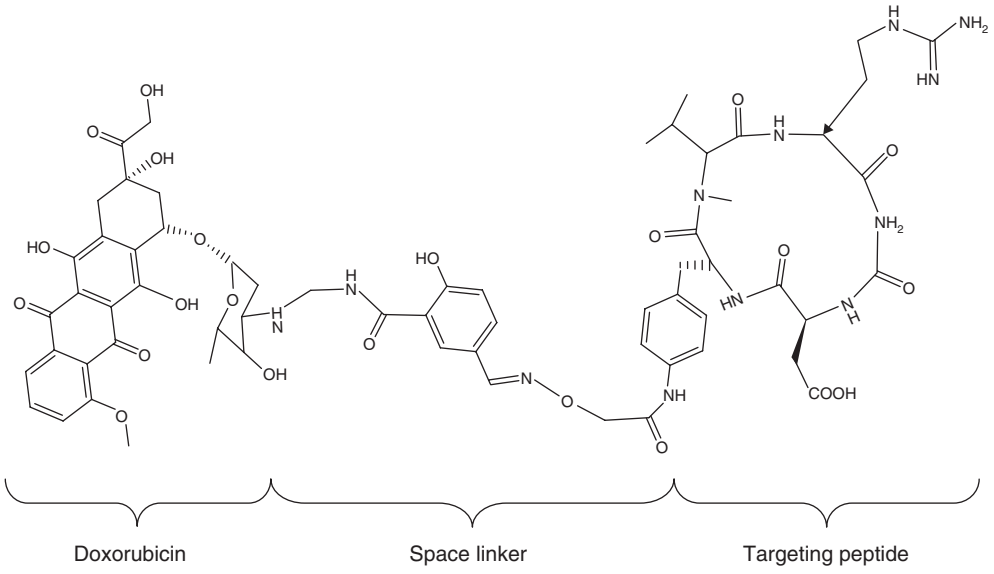

Figure 3. Doxorubicin–peptide conjugate with formaldehyde space linker.

years, Jain proposed that certain antiangiogenetic agents can transiently normalize the abnormal structure and function of the tumor vasculature, and make it more efficient for oxygen, nutrients and drug delivery [64,65]. Thus, combination of vascular targeting antagonists with conventional chemotherapeutical agents can facilitate the penetration of drug into tumor, as well as diminish the notorious side effects arising from cytotoxic drugs. Generally, there are two classes of molecules that could be conjugated with integrin antagonists: chemotherapeutical small molecules; and biomacromolecules.

It is well known that the conventional chemotherapeutical drugs are effective in their anticancer activity. However, their high toxicity to healthy cells limits their clinical efficacy. Thus, vectorization of the cytotoxic drugs only to the targeted cancer cells is believed to be a feasible approach to circumvent this side effect to some degree. For example, Arap *et al.* coupled the tumor vascular homing peptides (both targeting to integrin α_V) GDCFC (RGD4C) and CNGRCVSGCAGRC (NGR) to doxorubicin, a widely used anticancer drug for a variety of cancer treatments [66]. The result showed that the integrin targeting peptide–doxorubicin conjugate resulted in inhibition of tumor growth and fewer pulmonary metastases in mice than free doxorubicin. In addition, the conjugated doxorubicin was found to be less toxic to liver and heart than the free drug. In a similar manner, Kim and Lee also showed that RGD4C-conjugated doxorubicin was able to inhibit the tumor growth in $\alpha_V\beta_3$ integrin-negative tumor cell, suggesting that the antiangiogenetic effect on the endothelial cells induced by this conjugation may lead to tumor recession [67]. However, the peptide RGD4C contains disulfide bonds in its structure, which are susceptible to hydrolysis under reduced condition in cytoplasm, and result in inactive substance. Cyclic pentapeptide c(RGDfK) (Cilengitide) has emerged as an alternative to RGD4C for its good affinity and better water solubility than RGD4C. The *in vitro* cytotoxicity study revealed good growth inhibition of the breast cancer cell MDA-MB-435 (overexpressing $\alpha_V\beta_3$ integrin) (**Figure 3**) [68]. Besides doxorubicin, another front-line anticancer drug, paclitaxel, was also conjugated with integrin targeting bicyclic peptide $E[c(RGDyK)]_2$ [69,70]. The activity of the conjugate was evaluated in metastatic breast cancer cell MDA-MB-435 and found to be comparable to the free paclitaxel in the cancer cell proliferation inhibition assay. cIBR and cLABL have also been conjugated to methotrexate (MTX) to target to LFA-1 integrin [15].

Integrin ligands can also be conjugated with therapeutical biomacromolecules to target them to the specific intracellular site for their action. The conjugate of the bicyclic (ACDCRGDCFC) peptide with the antimicrobial synthetic peptide $(KLAKLAK)_2$ showed significant tumor-reducing effect and lowering in the lung metastasis burden [71]. Another naturally occurring antimicrobial peptide also showed antitumor efficacy on B16 melanoma-bearing syngenic mice after being coupled with integrin targeting peptide (RGD) [72]. RGD peptide was also used to couple to endostatin, an endogenous antiangiogenetic protein, for more effective tumor inhibition [73]. The cytokine protein TNF-α was shown to have better TNF receptor binding affinity and trigger apoptosis cascades after decorating with RGD integrin targeting ligand [74,75]. Using fusion protein technology, IL-12, IL-24, tTF (truncated tissue factor), or other tumor-suppressing and/or apoptosis-inducing cytokines have also been fused with different integrin targeting ligands for antitumor and antiangiogenesis activity [76-78].

4. Integrin targeted nanoparticle for drug delivery

A nanoparticulate delivery system with surface modified with integrin targeting ligand is another approach to delivering therapeutic agents to targeted cancer cells or diseased organs. Meanwhile, the nanoparticulate delivery system is believed to increase the stability of the encapsulated or conjugated therapeutic molecules, improve the efficacy, and alleviate the undesired side effecst. Also, as the tumor has leaky blood vessels and poor lymphatic drainage, nanoparticles can penetrate and accumulate in the tumor via those leaky vessels by the enhanced penetration and retention (EPR) effect [79]. To target receptor overexpressing cancers or disorder cells specifically, there are generally two classes of targeting: active targeting and passive targeting. Active targeting is programmed by engineering nanoparticles with cellular specific targeting moieties, although it is difficult to realize without passive targeting, in which poly(ethylene glycol) (PEG) is normally chosen to attenuate the surrounding environment (commonly aqueous or body fluid) to the nanoparticles to protect them from protein absorption and also to prolong the circulation time after administration into the body [80]. The most investigated nanoparticulate delivery systems are liposomes, polymeric nanospheres, micelles and polymersomes, with a broad size range from several tens of nanometers to hundreds of micrometers.

Among the integrin targeting ligands, the RGD-containing peptide-decorated nanoparticulate delivery system has been investigated most extensively, and fruitful results have been achieved. For example, RGD peptide was conjugated to the phospholipids with PEG as linker. The construct was then incorporated into liposome to realize both passive and active targeting functions. Murphy and co-workers had coupled cRGDfK peptide to PEGylated liposome encapsulating anticancer drug (doxorubicin) to target $\alpha_V\beta_3$ integrin-expressing tumor vasculature. In two different animal models, this $\alpha_V\beta_3$ integrin targeting-mediated delivery system resulted in a 15-fold increase in antimetastatic activity without any pronounced drug-associated weight loss as observed with systemic administration of the free drug [81]. Xiong *et al.* achieved high tumor accumulation and intercellular delivery of doxorubicin after conjugating synthetic RGD mimic compound with the drug-loading liposome in syngenic B16 melanoma mouse model. Administration of RGD mimic-modified nanoparticle resulted in retarded tumor growth and prolonged lifespan compared with the non-modified one [82,83]. Similar RGD liposome modification strategies have also been used to deliver other anticancer drugs (e.g., 5-FU, paclitaxel) to malignant tumor-bearing animals, and significant anti-primary tumor and antimetastatic activities were achieved [84-86].

RGD-decorated liposome was also used in combination therapy in which vascular disrupting agent (combretastatin)-loaded RGD surface-tethered liposome was combined with radiation therapy [87]. This combination therapy resulted in lower drug dosage (from 80 mg/kg of free drug to 14.5 mg/kg equivalent in the drug-loaded targeting nanoparticle) and better treatment outcome. Not just limited to cancer therapy, RGD-modified liposomes have also been applied for other disease treatments, and good therapeutic results achieved [88,89]. In addition to liposome, Nasongkla *et al.* developed biodegradable polymeric micelles, a type of self-assembled nanoparticle from amphiphilic copolymers, with cRGD surface functionalization for targeted doxorubicin delivery [90]. Later on, they made this delivery system multifunctional by adding iron oxide nanoparticles to the system for simultaneous MRI imaging and drug delivery [91].

Other integrin targeting micelle drug delivery system with diverse compositions are also available [92,93]. Biodegradable nanosphere is another attractive drug delivery system. Recently, an RGD-PEG-PLGA nanosphere delivery system was developed for intracellular delivery of doxorubicin to different malignant cancer cells (**Figure 4**) [80]. This new nanosphere possesses both passive and active targeting functions, and alleviates the burst drug release effect commonly associated with PLGA nanosphere systems. Besides using RGD as an integrin targeting ligand, Kim and co-workers also encapsulated RGD-containing peptide in nanoparticle delivery system for antitumor therapy [94]. In an *in vivo* study, the antiangiogenic peptide drug formulation of RGD–5β-cholanic acid (HGC) markedly inhibited bFGF-induced angiogenesis and decreased hemoglobin content in Matrigel plugs. Intratumoral administration of RGD–HGC significantly decreased tumor growth and microvessel density compared with native RGD peptide injected either intravenously or intratumorally. Swenson *et al.* reported the liposomal delivery of a new snake venom disintegrin, contortrostatin (CN) in an orthotopic human breast tumor xenograft model [95]. This disintegrin modulates its interaction with integrins on tumor cells and angiogenic vascular endothelial cells. By incorporating CN into a liposomal delivery system, several advantages were identified: i) CN in the liposomal delivery system had a significantly prolonged circulatory half-life compared with native CN; ii) CN in liposome was passively accumulated in the tumor; iii) it had no platelet reactivity; and iv) it was not recognized by the immune system. In their study, they demonstrated that intravenous delivery of liposomal CN led to potent antiangiogenic activity in the *in vivo* tumor model.

5. Integrin targeted nanoparticle for gene delivery

Nucleic acid is another attractive therapeutic agent for regulation of disorders or treatment of diseases including cancer, by substituting the defective genes, replacing the missing genes or silencing the undesired genes. Nucleic acid is hydrophilic, negatively charged and susceptible to enzyme degradation, its delivery *in vivo* depends much on loading it into a suitable delivery system for intracellular

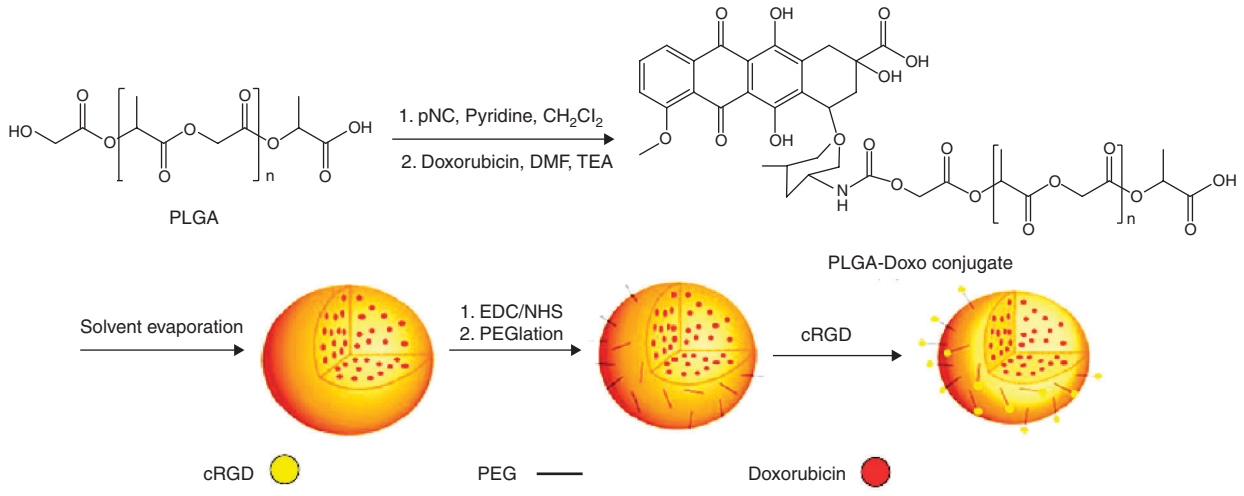

Figure 4. Scheme of multifunctional PLGA integrin targeting nanoparticle for controlled release of anticancer drug.
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delivery. In 2002, Hood *et al.* synthesized an $\alpha_V\beta_3$ integrin antagonist that was coupled with cationic lipids to facilitate gene delivery to angiogenic blood vessels in tumor-bearing mice (**Figure 5**). The fabricated nanoparticle was conjugated with a mutant *Raf* gene, which blocked endothelial signaling and angiogenesis stimulated by multiple growth factors. In a tumor-bearing mouse model, it was proved that the $\alpha_V\beta_3$ integrin targeting gene delivery nanoparticle was effective at inducing apoptosis in the tumor endothelial cells, ultimately leading to primary and metastatic tumor regression [96]. Similarly, an RGD sequence-containing peptide was also coupled with phospholipid to form a new lipopeptide that efficiently targeted β-galactosidase gene to tumor vasculature [97].

Polyethylenimine (PEI) is a commonly used effective non-virus cationic gene delivery vehicle. It is able to bind the negatively charged gene tightly and homogeneously. After coupling with RGD peptide, this delivery system facilitated luciferase plasmid DNA delivery into cancer cells, whereas mutated RGD peptide failed to do so [98]. To increase the stability further and prolong the circulation time *in vivo*, PEI was modified with PEG, followed by conjugation with RGD peptide at the distal end [99,100].

Ever since the discovery of the silencing effect of RNAi, delivery of siRNA into cells effectively to silence an unwanted gene has drawn much attention. Schiffelers and co-workers developed a self-assembled RGD surface-modified PEGylated PEI cationic nanoparticle to deliver siRNA against vascular endothelial growth factor receptor-2 (VEGFR-2) expression in human breast cancer-bearing mice [101]. This siRNA delivery nanoparticle inhibited the protein expression level and decreased the tumor growth and angiogenesis rate. siRNA delivery was also realized by conjugation of siRNA with albumin, which was decorated with RGD-conjugated PEG on the surface. The conjugates robustly induced luciferase expression at low concentrations, at which level little short-term or long-term toxicity was observed [102]. Harbottle *et al.* made it possible to use 16-lysine residue tethered integrin targeting peptide to condense DNA directly and deliver the DNA into cells. The delivering ability of this cationic peptide was reported to exceed the commercial Lipofectamine® (Invitrogen, CA) [103] reagent. On the other hand, siRNA delivery was used to silence specific integrin subunit expressions that were highly involved in mediation of cancer cell invasion, migration, metastasis, or hypoxia induction in tumor [104-106].

Apart from its application in cancer therapy, integrin targeted gene delivery has been used extensively for the treatment of other diseases, such as inflammation [107], HIV infection prevention [108], diabetes [109], and so on. Peer and co-workers have recently reported that integrin α_7 antibody-modified liposome loaded with cyclin D1 (CyD1)-specific siRNA can be systematically delivered to target specific leukocyte subsets involved in gut inflammation *in vivo*. On systemic administration, CyD1expression in leukocytes was successfully silenced, and this delivery system also reversed experimentally induced colitis in mice by suppressing leukocyte proliferation and T helper cell 1 cytokine expression [110]. Furthermore, adenovirus [111], liposomes [85,112], or polymeric nanoparticles [113,114] have also been used for integrin targeted gene delivery applications.

6. Conclusion

As one important member of the cell adhesion molecule family, integrin is highly associated with many human diseases and cellular function disorders by either single subunit or multiple subunits simultaneously. Herein, the most recent advances in integrin-based drug or gene delivery systems have been summarized. As a therapeutic target, there are many integrin antagonists, including antibodies, synthetic natural

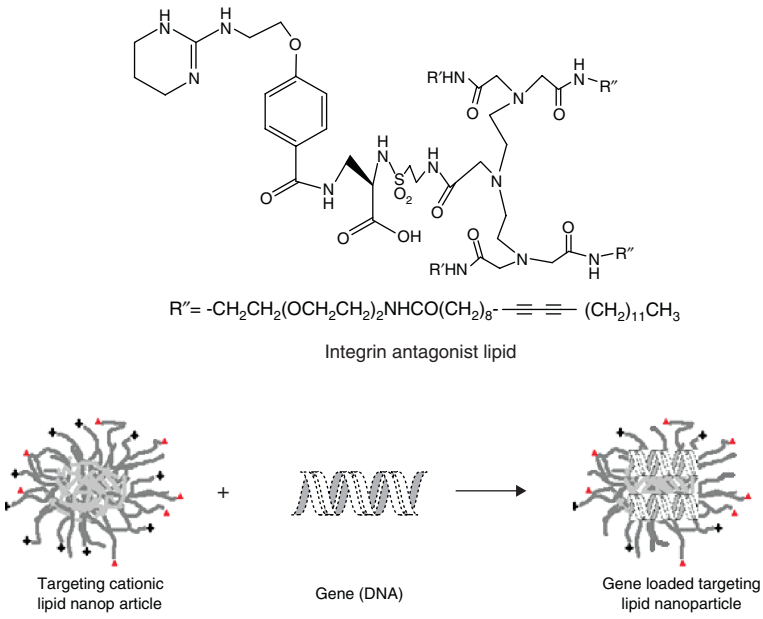

Figure 5. Scheme of integrin targeted gene delivery to neovasculature.

mimic peptides, naturally extracted disintegrins, peptidomimetics and other small molecules. Some of the antagonists are already on the market, and some are still being tested in different phases of clinical trials. Besides using them as therapeutic medicines, a fraction of the antagonists are used as integrin targeting ligands to direct drug or gene-loaded vehicles to specific abnormal cells. In this manner, the side effects of the cytotoxic drugs can be alleviated, to some extent, as well as lower doses of drugs being used for equivalent, if not better, therapeutic effect. Thus, the integrin targeted drug or gene delivery systems hold great promise for the treatment of diseases in human healthcare.

7. Expert opinion

With the rapid advance in modern technology, medicine, and our greater understanding of the complex biological signaling cascades and protein structures, researchers are now able to develop different bioactive moieties, including antibodies, natural mimic peptides, synthetic small molecules or nucleic acid, to fight against human diseases ranging from ailments such as fever to serious diseases such as cancer, AIDS, and so on. For the integrin targeted drug discovery, although so far only 24 heterodimers of integrins have been identified, researchers have developed hundreds of integrin antagonists targeting to different integrin heterodimers or functions. Many promising lead compounds or biomolecules are now being tested at different stages of clinical trials or are already available on the market. In the arena of cancer, as different integrins are present in various tumor types and are differentially expressed during tumor transformation, progression, invasion and metastasis, these suggest that integrins can also be utilized as diagnostic biomarkers.

In addition, integrin antagonists play essential roles as integrin targeting ligands to direct conventional therapeutic drugs to the targeted disorder sites in the development of the nanoparticle-based therapeutic delivery systems. Benefiting from the great development of nanomedicine, researchers have achieved significant improvements in the therapeutic efficacy with the integrin-directed drug delivery systems over the free drugs in terms of lower cytotoxicity and higher therapeutic effects. As many drug candidates have inherent limitations, including low bioavailability of many small molecules, susceptibility to enzyme degradation and low stability associated with biomolecules or nucleic acids, formulation of these drugs or genes into nanoparticles modified with integrin targeting ligands makes them more efficient at accessing the diseased cells and more efficacious at ultimately eliminating these cells.

Unfortunately, there still exist many challenges for the integrin targeted drug or gene delivery. First, the integrin antagonists that are available on the market or are in the late stage of clinical trials are still very limited in numbers. Second, formulation of drugs or genes into integrin targeting nanoparticles makes the manufacturing process very complicated, leading to a high barrier to clinical trials and eventual commercial uses. Thus, the formulation procedures must be kept as simple as possible. Meanwhile, the excipients used must be biocompatible and safe. Last but not least, the integrin targeted drugs and genes should be used extremely carefully for pregnant women and children, as there is concern that newborn tissues or organs have extensive neovascular networks, with overexpressed integrins as regulators. After all,

although there remain many uncertain questions and challenges, integrin targeted drug and gene delivery constitutes a formidable armament for the detection and treatment of various diseases for the better health of mankind.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Keibel A, Singh V, Sharma MC. Inflammation, microenvironment, and the immune system in cancer progression. Curr Pharm Des 2009;15(17):1949-55
2. Pavalko FM, Otey CA. Role of adhesion molecule cytoplasmic domains in mediating interactions with the cytoskeleton. Proc Soc Exp Biol Med 1994;205(4):282-93
3. Christofori G. Changing neighbours, changing behaviour: cell adhesion molecule-mediated signalling during tumour progression. EMBO J 2003;22(10):2318-23
4. Vyas SP, Vaidya B. Targeted delivery of thrombolytic agents: role of integrin receptors. Expert Opin Drug Deliv 2009;6(5):499-508
5. Andrews RK, Berndt MC. Platelet physiology and thrombosis. Thromb Res 2004;114(5-6):447-53
6. Alvarez-Rodriguez L, Ruiz M, Marin MJ, et al. Adhesion molecules expression and chemotactic ability of peripheral blood leukocytes in polymyalgia rheumatica and giant cell arteritis. Arthritis Rheum 2008;58(9):S394-5
7. Banquy X, Leclair G, Rabanel JM, et al. Selectins ligand decorated drug carriers for activated endothelial cell targeting. Bioconjug Chem 2008;19(10):2030-9
8. Ichinose K, Kawasaki E, Eguchi K. Recent advancement of understanding pathogenesis of type 1 diabetes and potential relevance to diabetic nephropathy. Am J Nephrol 2007;27(6):554-64
9. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002;110(6):673-87
10. Giancotti FG, Ruoslahti E. Transduction - integrin signaling. Science 1999;285(5430):1028-32
11. Hood JD, Cheresh DA. Role of integrins in cell invasion and migration. Nat Rev Cancer 2002;2(2):91-100
12. Eble JA, Haier J. Integrins in cancer treatment. Curr Cancer Drug Targets 2006;6(2):89-105
13. Sastry SK, Burridge K. Focal adhesions: a nexus for intracellular signaling and cytoskeletal dynamics. Exp Cell Res 2000;261(1):25-36
14. Burridge K, Wodnicka MC. Focal adhesions, contractility, and signaling. Annu Rev Cell Dev Biol 1996;12:463-518
15. Dunehoo AL, Anderson M, Majumdar S, et al. Cell adhesion molecules for targeted drug delivery. J Pharm Sci 2006;95(9):1856-72
16. Hynes RO. Integrins - versatility, modulation, and signaling in cell-adhesion. Cell 1992;69(1):11-25
17. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration - the multistep paradigm. Cell 1994;76(2):301-14
18. Curley GP, Blum H, Humphries MJ. Integrin antagonists. Cell Mol Life Sci 1999;56(5-6):427-41
19. Shimaoka M, Springer TA. Therapeutic antagonists and conformational regulation of integrin function. Nat Rev Drug Discov 2003;2(9):703-16
20. Coller BS. Platelet GPIIb/IIIa antagonists: The first anti-integrin receptor therapeutics. J Clin Invest 1997;100(11):S57-60
21. Trikha M, Zhou Z, Timar J, et al. Multiple roles for platelet GPIIb/IIIa and alpha v beta 3 integrins in tumor growth, angiogenesis, and metastasis. Cancer Res 2002;62(10):2824-33
22. Tam SH, Sassoli PM, Jordan RE, Nakada MT. Abciximab (ReoPro, chimeric 7E3 Fab) demonstrates equivalent affinity and functional blockade of glycoprotein IIb/IIIa and alpha(v)beta (3) integrins. Circulation 1998;98(11):1085-91
23. Brooks PC, Clark RAF, Cheresh DA. Requirement of vascular integrin alpha(V) beta(3) for angiogenesis. Science 1994;264(5158):569-71
24. Nakada MT, Cao G, Sassoli PM, DeLisser HM. c7E3 Fab inhibits human tumor angiogenesis in a SCID mouse human skin xenograft model. Angiogenesis 2006;9(4):171-6
25. Califf RM, Shadoff N, Valett N, et al. Use of a monoclonal-antibody directed against the platelet glycoprotein Iib/Iiia receptor in high-risk coronary angioplasty. N Engl J Med 1994;330(14):956-61
26. Topol EJ, Califf RM, Weisman HF, et al. Randomized trial of coronary intervention with antibody against platelet Iib/Iiia integrin for reduction of clinical restenosis - results at 6 months. Lancet 1994;343(8902):881-6
27. Cheresh DA. Human-endothelial cells synthesize and express an arg-gly-asp-directed adhesion receptor involved in attachment to fibrinogen and vonwillebrand-factor. Proc Natl Acad Sci USA 1987;84(18):6471-5
28. Friedlander M, Brooks PC, Shaffer RW, et al. Definition of 2 angiogenic pathways by distinct alpha(V) integrins. Science 1995;270(5241):1500-2
29. Brooks PC, Stromblad S, Klemke R, et al. Antiintegrin alpha-v-beta-3 blocks human breast-cancer growth and angiogenesis in human skin. J Clin Invest 1995;96(4):1815-22
30. Brooks PC, Montgomery AMP, Rosenfeld M, et al. Integrin alpha-v-beta-3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell 1994 1994;79(7):1157-64
31. Rader C, Popkov M, Neves JA, Barbas CF. Integrin alpha v beta 3-targeted therapy for Kaposi's sarcoma with an in vitro-evolved antibody. FASEB J 2002;16(12):2000
32. Posey JA, Khazaeli MB, DelGrosso A, et al. A pilot trial of vitaxin, a humanized anti-vitronectin receptor (anti alpha(v)beta (3)) antibody in patients with metastatic cancer. Cancer Biother Radiopharm 2001;16(2):125-32
33. Liu ZF, Wang F, Chen XY. Integrin alpha (v)beta(3)-targeted cancer therapy. Drug Dev Res 2008;69(6):329-39

34. Trikha M, Zhou Z, Nemeth JA, et al. CNTO 95, a fully human monoclonal antibody that inhibits alpha v integrins, has antitumor and antiangiogenic activity in vivo. Int J Cancer 2004;110(3):326-35

35. Chen QM, Manning CD, Millar H, et al. CNTO 95, a fully human anti alpha v integrin antibody, inhibits cell signaling, migration, invasion, and spontaneous metastasis of human breast cancer cells. Clin Exp Metastasis 2008;25(2):139-48

36. Mullamitha SA, Ton NC, Parker GJM, et al. Phase I evaluation of a fully human anti-alpha(v) integrin monoclonal antibody (CNTO 95) in patients with advanced solid tumors. Clin Cancer Res 2007;13(7):2128-35

37. Ning SC, Nemeth JA, Hanson RL, et al. Anti-integrin monoclonal antibody CNTO 95 enhances the therapeutic efficacy of fractionated radiation therapy in vivo. Mol Cancer Ther 2008;7(6):1569-78

38. Mitjans F, Sander D, Adan J, et al. An anti-alpha-v-integrin antibody that blocks integrin function inhibits the development of a human-melanoma in nude-mice. J Cell Sci 1995;108:2825-38

39. Mitjans F, Meyer T, Fittschen C, et al. In vivo therapy of malignant melanoma by means of antagonists of alpha v integrins. Int J Cancer 2000;87(5):716-23

40. Ruoslahti E, Pierschbacher MD. Arg-gly-asp - a versatile cell recognition signal. Cell 1986;44(4):517-8

41. Marinelli L, Lavecchia A, Gottschalk KE, et al. Docking studies on alpha(v)beta(3) integrin ligands: pharmacophore refinement and implications for drug design. J Med Chem 2003;46(21):4393-404

42. Kopple KD, Baures PW, Bean JW, et al. Conformations of arg-gly-asp containing heterodetic cyclic-peptides - solution and crystal studies. J Am Chem Soc 1992;114(24):9615-23

43. Dechantsreiter MA, Planker E, Matha B, et al. N-methylated cyclic RGD peptides as highly active and selective alpha(v)beta(3) integrin antagonists. J Med Chem 1999;42(16):3033-40

44. MacDonald TJ, Stewart CF, Kocak M, et al. Phase I clinical trial of cilengitide in children with refractory brain tumors: pediatric brain tumor consortium study PBTC-012. J Clin Oncol 2008;26(6):919-24

45. Eskens F, Dumez H, Hoekstra R, et al. Phase I and pharmacokinetic study of continuous twice weekly intravenous administration of cilengitide (EMD 121974), a novel inhibitor of the integrins alpha v beta 3 and alpha v beta 5 in patients with advanced solid tumours. Eur J Cancer 2003;39(7):917-26

46. Beekman KW, Colevas AD, Cooney K, et al. Phase II evaluations of cilengitide in asymptomatic patients with androgen-independent prostate cancer: Scientific rationale and study design. Clin Genitourin Cancer 2006;4(4):299-302

47. Friess H, Langrehr JM, Oettle H, et al. A randomized multi-center phase II trial of the angiogenesis inhibitor cilengitide (EMD 121974) and gemcitabine compared with gemcitabine alone in advanced unresectable pancreatic cancer. BMC Cancer 2006;6:285

48. Nabors LB, Mikkelsen T, Rosenfeld SS, et al. Phase I and correlative biology study of cilengitide in patients with recurrent malignant glioma. J Clin Oncol 2007;25(13):1651-7

49. Khalili P, Arakelian A, Chen GP, et al. A non-RGD-based integrin binding peptide (ATN-161) blocks breast cancer growth and metastasis in vivo. Mol Cancer Ther 2006;5(9):2271-80

50. Livant DL, Brabec RK, Pienta KJ, et al. Anti-invasive, antitumorigenic, and antimetastatic activities of the PHSCN sequence in prostate carcinoma. Cancer Res 2000;60(2):309-20

51. Stoeltzing O, Liu WB, Reinmuth N, et al. Inhibition of integrin alpha(5)beta(1) function with a small peptide (ATN-161) plus continuous 5-FU infusion reduces colorectal liver metastases and improves survival in mice. Int J Cancer 2003;104(4):496-503

52. Donate F, Parry GC, Shaked Y, et al. Pharmacology of the novel antiangiogenic peptide ATN-161 (Ac-PHSCN-NH2): observation of a U-shaped dose-response curve in several preclinical models of angiogenesis and tumor growth. Clin Cancer Res 2008;14(7):2137-44

53. Cianfrocca ME, Kimmel KA, Gallo J, et al. Phase 1 trial of the antiangiogenic peptide ATN-161 (Ac-PHSCN-NH2), a beta integrin antagonist, in patients with solid tumours. Br J Cancer 2006;94(11):1621-6

54. Thibault G, Tardif P, Lapalme G. Comparative specificity of platelet alpha(IIb)beta(3) integrin antagonists. J Pharmacol Exp Ther 2001;296(3):690-6

55. Lal H, Guleria RS, Foster DM, et al. Integrins: novel therapeutic targets for cardiovascular diseases. Cardiovasc Hematol Agents Med Chem 2007;5(2):109-32

56. Yusuf-Makagiansar H, Makagiansar IT, Hu YB, Siahaan TJ. Synergistic inhibitory activity of alpha- and beta-LFA-1 peptides on LFA-1/ICAM-1 interaction. Peptides 2001;22(12):1955-62

57. Tibbetts SA, Chirathaworn C, Nakashima M, et al. Peptides derived from ICAM-1 and LFA-1 modulate T cell adhesion and immune function in a mixed lymphocyte culture. Transplantation 1999;68(5):685-92

58. Yusuf-Makagiansar H, Siahaan TJ. Binding and internalization of an LFA-1-derived cyclic peptide by ICAM receptors on activated lymphocyte: a potential ligand for drug targeting to ICAM-1-expressing cells. Pharm Res 2001;18(3):329-35

59. Lu X, Lu D, Scully MF, Kakkar VV. Integrins in drug targeting-RGD templates in toxins. Curr Pharm Des 2006;12(22):2749-69

- **A summary of RGD templated disintegrins and their interaction mechanisms.**

60. McLane MA, VijayKumar S, Marcinkiewicz C, et al. Importance of the structure of the RGD-containing loop in the disintegrins echistatin and eristostatin for recognition of alpha IIb beta 3 and alpha v beta 3 integrins. FEBS Lett 1996;391(1-2):139-43

61. Okuda D, Koike H, Morita T. A new gene structure of the disintegrin family: a subunit of dimeric disintegrin has a short coding region. Biochemistry 2002;41(48):14248-54

62. Huang TF, Holt JC, Lukasiewicz H, Niewiarowski S. Trigramin - a low-molecular-weight peptide inhibiting fibrinogen interaction with platelet receptors expressed on glycoprotein-Iib-Iiia complex. J Biol Chem 1987;262(33):16157-63

63. Horton DA, Bourne GT, Smythe ML. The combinatorial synthesis of bicyclic privileged structures or privileged

substructures. Chem Rev 2003;103(3):893-930

64. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. Nat Med 2001;7(9):987-9

65. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005;307(5706):58-62

•• **References [64] and [65] are important articles describing the impact on normalizing tumor vasculature in cancer therapy.**

66. Arap W, Pasqualini R, Ruoslahti E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science 1998;279(5349):377-80

67. Kim JW, Lee HS. Tumor targeting by doxorubicin-RGD-4C peptide conjugate in an orthotopic mouse hepatoma model. Int J Mol Med 2004;14(4):529-35

68. Burkhart DJ, Kalet BT, Coleman MP, et al. Doxorubicin-formaldehyde conjugates targeting alpha(v)beta(3) integrin. Mol Cancer Ther 2004;3(12):1593-604

69. Chen XY, Plasencia C, Hou YP, Neamati N. Synthesis and biological evaluation of dimeric RGD peptide-paclitaxel conjugate as a model for integrin-targeted drug delivery. J Med Chem 2005;48(4):1098-106

70. Cao QZ, Li ZB, Chen K, et al. 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 2008;35(8):1489-98

71. Ellerby HM, Arap W, Ellerby LM, et al. Anti-cancer activity of targeted pro-apoptotic peptides. Nat Med 1999;5(9):1032-8

72. Chen YX, Xu XM, Hong SG, et al. RGD-tachyplesin inhibits tumor growth. Cancer Res 2001;61(6):2434-8

73. Yokoyama Y, Ramakrishnan S. Addition of integrin binding sequence to a mutant human endostatin improves inhibition of tumor growth. Int J Cancer 2004;111(6):839-48

74. Curnis F, Gasparri A, Sacchi A, et al. Coupling tumor necrosis factor-alpha with alpha(v) integrin ligands improves its antineoplastic activity. Cancer Res 2004;64(2):565-71

75. Sacchi A, Gasparri A, Curnis F, et al. Crucial role for interferon gamma in the synergism between tumor vasculature-targeted tumor necrosis factor alpha (NGR-TNF) and doxorubicin. Cancer Res 2004;64(19):7150-5

76. Xiao B, Li W, Yang J, et al. RGD-IL-24, a novel tumor-targeted fusion cytokine: expression, purification and functional evaluation. Mol Biotechnol 2009;41(2):138-44

77. Bieker R, Kessler T, Schwoppe C, et al. Infarction of tumor vessels by NGR-peptide-directed targeting of tissue factor: experimental results and first-in-man experience. Blood 2009;113(20):5019-27

78. Dickerson EB, Akhtar N, Steinberg H, et al. Enhancement of the antiangiogenic activity of interleukin-12 by peptide targeted delivery of the cytokine to alpha (v)beta(3) integrin. Mol Cancer Res 2004;2(12):663-73

79. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer-chemotherapy - mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 1986;46(12):6387-92

80. Wang Z, Chui WK, Ho PC. Design of a multifunctional PLGA nanoparticulate drug delivery system: evaluation of its physicochemical properties and anticancer activity to malignant cancer cells. Pharm Res 2009;26(5):1162-71

81. Murphy EA, Majeti BK, Barnes LA, et al. Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis. Proc Natl Acad Sci USA 2008;105(27):9343-8

82. Xiong XB, Huang Y, Lu WL, et al. Enhanced intracellular delivery and improved antitumor efficacy of doxorubicin by sterically stabilized liposomes modified with a synthetic RGD mimetic. J Control Release 2005;107(2):262-75

83. Xiong XB, Huang Y, Lu WL, et al. Intracellular delivery of doxorubicin with RGD-modified sterically stabilized liposomes for an improved antitumor efficacy: in vitro and in vivo. J Pharm Sci 2005;94(8):1782-93

• **An earlier study described the improvement in intracellular drug delivery and tumor growth reduction by conjugating synthetic RGD to the drug-loading liposome.**

84. Dubey PK, Mishra V, Jain S, et al. Liposomes modified with cyclic RGD peptide for tumor targeting. J Drug Target 2004;12(5):257-64

85. Holig P, Bach M, Volkel T, et al. Novel RGD lipopeptides for the targeting of liposomes to integrin-expressing endothelial and melanoma cells. Protein Eng Des Sel 2004;17(5):433-41

86. Zhao H, Wang JC, Sun QS, et al. RGD-based strategies for improving antitumor activity of paclitaxel-loaded liposomes in nude mice xenografted with human ovarian cancer. J Drug Target 2009;17(1):10-8

87. Pattillo CB, Sari-Sarraf F, Nallamothu R, et al. Targeting of the antivascular drug combretastatin to irradiated tumors results in tumor growth delay. Pharm Res 2005;22(7):1117-20

88. Sen Gupta A, Huang G, Lestini BJ, et al. RGD-modified liposomes targeted to activated platelets as a potential vascular drug delivery system. Thromb Haemost 2005;93(1):106-14

89. Lestini BJ, Sagnella SM, Xu Z, et al. Surface modification of liposomes for selective cell targeting in cardiovascular drug delivery. J Control Release 2002;78(1-3):235-47

90. Nasongkla N, Shuai X, Ai H, et al. cRGD-functionalized polymer micelles for targeted doxorubicin delivery. Angew Chem Int Ed 2004;43(46):6323-7

91. Nasongkla N, Bey E, Ren JM, et al. Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems. Nano Lett 2006;6(11):2427-30

92. Deng C, Chen XS, Sun J, et al. RGD peptide grafted biodegradable amphiphilic triblock copolymer poly(glutamic acid)-b-poly(L-lactide)-b-poly(glutamic acid): synthesis and self-assembly. J Poly Sci A Poly Chem 2007;45(15):3218-30

93. Shen SI, Kotamraj PR, Bhattacharya S, et al. Synthesis and characterization of RGD-fatty acid amphiphilic micelles as targeted delivery carriers for anticancer agents. J Drug Target 2007;15(1):51-8

94. Kim JH, Kim YS, Park K, et al. Self-assembled glycol chitosan nanoparticles for the sustained and prolonged delivery of antiangiogenic small peptide drugs in cancer therapy. Biomaterials 2008;29(12):1920-30

95. Swenson S, Costa F, Minea R, et al. Intravenous liposomal delivery of the snake venom disintegrin contortrostatin limits breast cancer progression. Mol Cancer Ther 2004;3(4):499-511

96. Hood JD, Bednarski M, Frausto R, et al. Tumor regression by targeted gene delivery to the neovasculature. Science 2002;296(5577):2404-7

97. Pramanik D, Majeti BK, Mondal G, et al. Lipopeptide with a RGDK tetrapeptide sequence can selectively target genes to proangiogenic alpha 5 beta 1 integrin receptor and mouse tumor vasculature. J Med Chem 2008;51(22):7298-302

•• **An interesting article describing the coupling of RGD sequence-containing peptide to phospholipid to form a new lipopeptide that efficiently targets beta-galactosidase gene to tumor vasculature.**

98. Erbacher P, Remy JS, Behr JP. Gene transfer with synthetic virus-like particles via the integrin-mediated endocytosis pathway. Gene Ther 1999;6(1):138-45

99. Kim WJ, Yockman JW, Lee M, et al. Soluble Flt-1 gene delivery using PEI-g-PEG-RGD conjugate for anti-angiogenesis. J Control Release 2005;106(1-2):224-34

100. Suh W, Han SO, Yu L, Kim SW. An angiogenic, endothelial-cell-targeted polymeric gene carrier. Mol Ther 2002;6(5):664-72

101. Schiffelers RM, Ansari A, Xu J, et al. Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle. Nucleic Acids Res 2004;32(19)

102. Kang H, Alam MR, Dixit V, et al. Cellular delivery and biological activity of antisense oligonucleotides conjugated to a targeted protein carrier. Bioconjug Chem 2008;19(11):2182-8

103. Harbottle RP, Cooper RG, Hart SL, et al. An RGD-oligolysine peptide: a prototype construct for integrin-mediated gene delivery. Hum Gene Ther 1998;9(7):1037-47

104. Skuli N, Monferran S, Delmas C, et al. alpha(v)beta(3)/alpha(v)beta(5) integrins-FAK-RhoB: a novel pathway for hypoxia regulation in glioblastoma. Cancer Res 2009;69(8):3308-16

105. Zhang XP, Fournier MV, Ware JL, et al. Inhibition of vimentin or beta(1) integrin revert morphology of prostate tumor cells grown in laminin-rich extracellular matrix gels and reduces tumor growth in vivo. Mol Cancer Ther 2009;8(3):499-508

106. Lipscomb EA, Dugan AS, Rabinovitz I, Mercurio AM. Use of RNA interference to inhibit integrin (alpha 6 beta 4)-mediated invasion and migration of breast carcinoma cells. Clin Exp Metastasis 2003;20(6):569-76

107. Peer D, Shimaoka M. Systemic siRNA delivery to leukocyte-implicated diseases. Cell Cycle 2009;8(6):853-9

•• **A recent article on integrin targeted and stabilized nanoparticles as a vehicle for delivering siRNA in the treatment of leukocyte-implicated disease.**

108. Kim SS, Peer D, Kumar P, et al. siRNA delivery with integrin LFA-1-targeted nanoparticles prevents hiv infection in humanized mice. Clin Immunol 2009;131:S75-6

109. Bhattacharyya J, Mondal G, Madhusudana K, et al. Single subcutaneous administration of RGDK-lipopeptide:rhPDGF-B gene complex heals wounds in streptozotocin-induced diabetic rats. Mol Pharm 2009;6(3):918-27

110. Peer D, Park EJ, Morishita Y, et al. Systemic leukocyte-directed siRNA delivery revealing cyclin D1 as an anti-inflammatory target. Science 2008;319(5863):627-30

111. Toh ML, Hong AS, van de Loo F, et al. Enhancement of adenovirus-mediated gene delivery to rheumatoid arthritis synoviocytes and synovium by fiber modifications: role of arginine-glycine-aspartic acid (RGD)and non-RGD-binding integrins. J Immunol 2005;175(11):7687-98

112. Harvie P, Dutzar B, Galbraith T, et al. Targeting of lipid-protamine-DNA (LPD) lipopolyplexes using RGD motifs. J Liposome Res 2003;13(3-4):231-47

113. Zugates GT, Anderson DG, Little SR, et al. Synthesis of poly(beta-amino ester)s with thiol-reactive side chains for DNA delivery. J Am Chem Soc 2006;128(39):12726-34

114. Green JJ, Chiu E, Leshchiner ES, et al. Electrostatic ligand coatings of nanoparticles enable ligand-specific gene delivery to human primary cells. Nano Lett 2007;7(4):874-9

Affiliation

Zhe Wang, Wai-Keung Chui & Paul C Ho†
†Author for correspondence
National University of Singapore,
Department of Pharmacy,
18 Science Drive 4,
Singapore 117543,
Singapore
Tel: +65 6516 2651; Fax: +65 6779 1554;
E-mail: phahocl@nus.edu.sg