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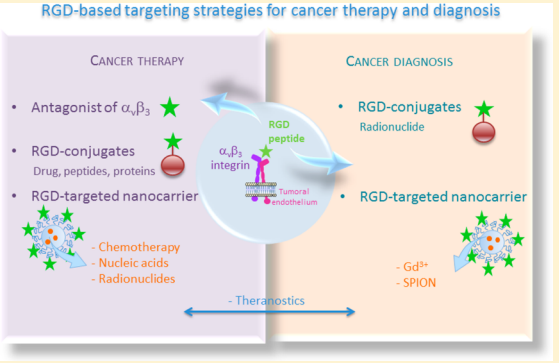
RGD-Based Strategies To Target Alpha(v) Beta(3) Integrin in Cancer Therapy and Diagnosis

Fabienne Danhier, Aude Le Breton, and Véronique Préat*

Université catholique de Louvain, Pharmaceutics and Drug Delivery, Louvain Drug Research Institute, Avenue E. Mounier, 73 B1 73 12, B-1200, Brussels, Belgium

ABSTRACT: The integrin $\alpha_v\beta_3$ plays an important role in angiogenesis. It is expressed on tumoral endothelial cells as well as on some tumor cells. RGD peptides are well-known to bind preferentially to the $\alpha_v\beta_3$ integrin. In this context, targeting tumor cells or tumor vasculature by RGD-based strategies is a promising approach for delivering anticancer drugs or contrast agents for cancer therapy and diagnosis. RGD-based strategies include antagonist drugs (peptidic or peptidomimetic) of the RGD sequence, RGD-conjugates, and the grafting of the RGD peptide or peptidomimetic, as targeting ligand, at the surface of nanocarriers. Although all strategies are overviewed, this review aims to particularly highlight the position of RGD-based nanoparticles in cancer therapy and imaging. This review is divided into three parts: the first one describes the context of angiogenesis, the role of the integrin $\alpha_v\beta_3$, and the binding of the RGD peptide to this integrin; the second one focuses on RGD-based strategies in cancer therapy; while the third one focuses on RGD-based strategies in cancer diagnosis.



KEYWORDS: *RGD, alpha(v) beta(3) integrin, nanoparticles, tumor vasculature, angiogenesis, cancer therapy, cancer diagnosis*

1. INTRODUCTION

1.1. Angiogenesis. Angiogenesis is a critical process involving the formation of new blood vessels from preexisting vessels. Normal angiogenesis is an essential process of fetal development, wound healing, ovulation, growth and development. In 1971, Judah Folkman was the first to hypothesize that solid tumors have limited resources for which the many actively proliferating cancer cells fight, initializing the beginning of the importance of angiogenesis in tumor growth.[1,2]

When tumors reach approximately 2 mm^3, the increased interstitial pressure within the tumor inhibits the diffusion of metabolites and nutrients necessary for tumor growth. A state of cellular hypoxia begins, inducing the sprouting of new blood vessels from the established vasculature. Consequently, oxygen and nutrients are carried to tumor cells, which need them to survive and proliferate.[3] Hypoxia increases cellular hypoxia inducible factor (HIF) transcription, leading to upregulation of proangiogenic proteins such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), or tumor necrosis factor-α (TNF-α).[4] New blood vessel formation begins with the removal of pericytes from pre-exisiting blood vessels, initiating the degradation of the endothelial cell basement membrane and extracellular matrix, a process regulated by the matrix metalloproteinases (MMPs). After this degradation, endothelial cells proliferate and migrate until they form unstable microvessels. Mesenchymal cells differentiate into pericytes, which allow the stability of new formatted vessels. Blood flow can then be established[2] (Figure 1). The progression of the tumor from a nonangiogenic to an angiogenic phenotype is called the "angiogenic switch". The angiogenic switch is triggered by signals such as metabolic stress (low pH, low oxygen pressure), mechanical stress, inflammatory response, and genetic mutations.[5] These signals lead to (i) increased expression of angiogenic proteins by tumor cells, such as VEGF; (ii) increased expression of angiogenic proteins by stromal cells; and (iii) decreased expression of angiogenic inhibitors such as thrombospondin-1 by tumor cells and stromal cells, which directly governed the angiogenic switch. During the "dormancy state", meaning before tumor angiogenesis, the tumor mass expands slowly, resulting in an asymptomatic and nonmetastatic state. However, after the angiogenic switch the tumor mass expands rapidly.[6]

Various classes of adhesion molecules are involved in tumor angiogenesis. Members of the integrin, cadherin, selectin, and immunoglobulin families participate in each step of tumor vascularization. The contribution of these cell adhesion molecules in tumor angiogenesis has been reviewed.[5,7] Because this review will discuss the RGD-based anticancer strategies, we will focus only on the $\alpha_v\beta_3$ integrin, which is particularly recognized by the RGD peptide.

1.2. $\alpha_v\beta_3$ Integrin. Among cell adhesion molecules (CAM), integrins are cell adhesion receptors for extracellular matrix (ECM) proteins, immunoglobulin, growth factors, cytokines,

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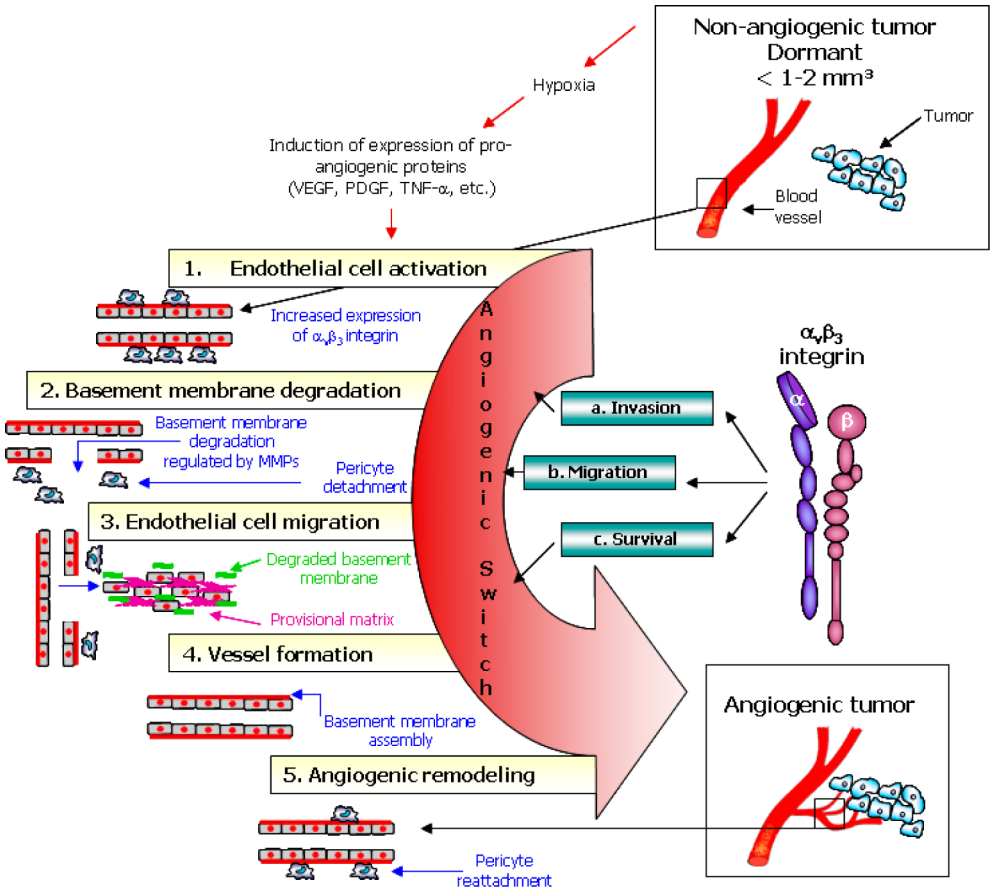

Figure 1. Roles of the $\alpha_v\beta_3$ integrin in angiogenesis. The $\alpha_v\beta_3$ integrin binds and activates MMP-2 to help break down the extracellular matrix. The $\alpha_v\beta_3$ integrin regulates cell attachment, spreading, and migration. In endothelial cells, ligated $\alpha_v\beta_3$ integrins prevent apoptosis through the intrinsic apoptosis pathway. Adapted from ref 2.

and matrix-degrading proteases. Integrins are divalent cation-dependent heterodimeric membrane glycoproteins composed of noncovalently associated α- and β-subunits. Eighteen α-subunits and 8 β-subunits can assemble into 24 different heterodimers. Each subunit is composed of (i) an extracellular domain, (ii) a single transmembrane region, and (iii) a cytoplasmic region.[8] The combination of α- and β-subunits determines the ligand binding specificity and signaling properties of a given integrin. Integrins can be classified based on their properties or based on their subunit composition, which are well presented in the review written by Barczyk et al.[9] Most integrins recognize their respective ECM proteins through short peptide sequences such as Arg-Gly-Asp (RGD), Glu-Ile-Leu-Asp-Val (EILDV), or Arg-Glu-Asp-Val (REDV).

Integrins are essential cell adhesion receptors. Integrin ligation promotes integrins clustering and induces intracellular signal transduction. After binding to its ECM protein (e.g., fibronectin and vitronectin) or cell surface immunoglobulin proteins (e.g., intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)), integrins (which have no intrinsic enzymatic or kinase activities) initiate a signaling cascade, which can include tyrosine phosphorylation of focal adhesion kinases (FAK).[10,11] Integrins not only signal on their own but also cooperate with growth factors receptors (GFRs) in regulating many cellular processes. It has been demonstrated that $\alpha_v\beta_3$ forms complexes with the insulin receptor, PDGF, and VEGF.[12]

Integrins are able to mediate adhesive events during various cancer stages such as malignant transformation, tumor growth and progression, invasion, and metastasis. The expression of β3 integrins is mostly associated with the ability of tumors to metastasize.[12] Tumor cells can migrate effectively on ECM substrates, and the multiple integrin functioning contributes to this process.[12] A wide variety of integrins are involved in angiogenesis. Among all integrins, $\alpha_v\beta_3$ is probably the most strongly involved in the regulation of angiogenesis. Its expression on endothelial cells is stimulated by angiogenic growth factors such as fibroblast growth factor-2 (FGF-2), TNF-α, and interleukin-8 (IL-8), which are upregulated in endothelium tumors, wounds and sites of inflammation.[8,13] The $\alpha_v\beta_3$ integrin localizes with proteolytically active MMP-2 on the surface of angiogenic blood vessels, resulting in cell-mediated collagen degradation and consequently the rearrangement of the ECM. Therefore it has been reported that, after the binding of $\alpha_v\beta_3$ integrin to fibronectin, fibrinogen, or osteopontin, the induction of endothelial cell migration is facilitated. Moreover, the level of COX-2 is regulated by the activation of $\alpha_v\beta_3$ integrin, which is required during endothelial cell spreading and migration. The $\alpha_v\beta_3$ integrin has also shown a prosurvival function: after the binding to fibronectin, endothelial cells are protected from apoptosis through the activation of different signaling cascades (Figure 1).[5]

Although not all integrins have the same extremes in activation potential, it is generally accepted that most integrins, including integrins expressed on endothelial cells, can have "on"

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and "off" states. The extracellular domain of $\alpha_v\beta_3$ integrin is bent or folded, thereby hiding the RGD-binding site and preventing ligand binding. Conversely, RGD-bound $\alpha_v\beta_3$ integrin has an unbent or straighter extracellular domain (Figure 2). Although integrin cytoplasmic tails are much

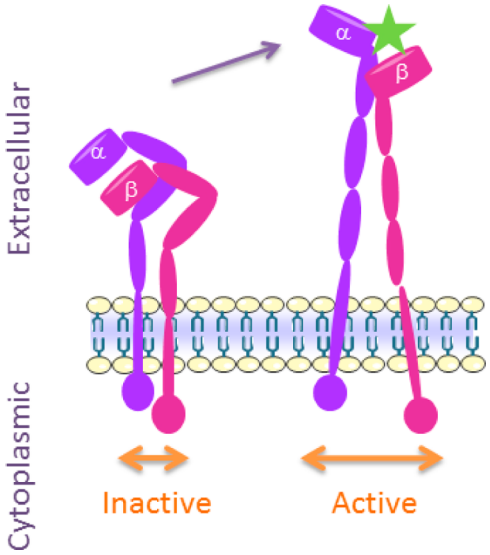

Figure 2. Conformational changes in $\alpha_v\beta_3$ integrin. Upon activation the extracellular domains extend and straighten, exposing the RGD-binding domain (star). Adapted from ref 14.

smaller than their extracellular domains, they can play pivotal roles in integrin signaling events, with separation, twisting, and hinging of the tails all considered mechanisms to allow activation.[14]

Integrins regulate multiple pathways that are constitutively activated in cancer cells, including Erk, FAK, Src, and Rho GTPases. Normal cells have multiple mechanisms to ensure that signaling is terminated. In contrast, cancer cells have lost their anchorage dependence and signaling pathways required for cell growth and survival are constitutively activated, bypassing the integrin requirement for regulated activation of these pathways.[15] It was suggested that $\beta1$ containing integrins are most likely involved in the clathrin-mediated pathway. Moreover, several studies have demonstrated that $\beta3$ integrin interact with caveolin-1, suggesting that raft/caveolar endocytosis (RCE) may also be an important pathway for internalization of this integrin. In cancer cells, $\beta3$ integrin block RCE of lipid rafts in order to maintain integrin-mediated cell signaling adherent cells. Loss of anchorage dependence of growth has been linked with tumor metastasis. Following cell detachment, lipid rafts are internalized rapidly via phosphorylated caveolin-1-mediated RCE, which mediates the inhibition of key integrin signaling molecules such as Erk, PI3K, and Rac. More particularly, because of the activity of $\alpha_v\beta_3$ integrin of tumor proteolytic enzymes (MMP-2), the alterations in integrin endocytosis induce modulation of proteolytic activity at the tumor cell surface, affecting cellular migration.[16]

Integrin cooperation with particular growth factor receptors seems to confer responsiveness to specific angiogenic growth factors that are highly expressed in tumors. For example, $\alpha_v\beta_3$ and FGF receptor interaction induces angiogenesis downstream of FGF binding, and $\alpha_v\beta_5$ and VEGF-2 receptor promote VEGF-induced angiogenesis. The development of cilengitide as an inhibitor of both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin was partly based on these findings. These distinct pathways of angiogenesis highlight the fact that integrins can integrate cues from the ECM and growth factors to drive specific intracellular signaling events.[7] Inhibitors of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin have been reported

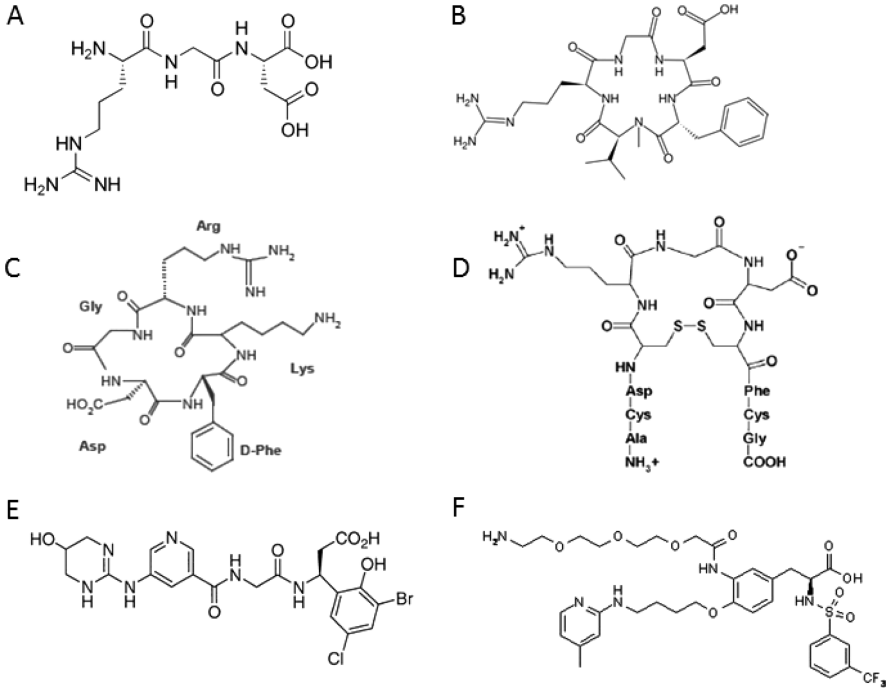

Figure 3. Chemical structures. (A) The original **RGD** sequence. (B) Cyclic RGD peptide antagonist (c(**RGD**f[N-Me]V) or cilengitide. (C) Cyclic peptide c(RGDfK). (D) ACDC**RGD**CFCG (RGD4C). (E) Example of RGD peptidomimetic-containing the RGD sequence (S-247).[28] (F) Example of RGD peptidomimetic.[29]

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to suppress pathological angiogenesis in mice. Conversely, the enhanced tumor growth and angiogenesis shown by β3 and β5 integrin doubly deficient mice strongly suggests that β5 integrin is actually not required for pathological angiogenesis.[17]

1.3. $\alpha_v\beta_3$ Integrin Expression in Cancer. $\alpha_v\beta_3$ integrin is widely expressed on blood vessels of human tumor biopsy samples but not on vessels in normal tissues. Tumor cell expression of the integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, $\alpha_6\beta_4$, $\alpha_4\beta_1$, and $\alpha_v\beta_6$ is correlated with disease progression in various tumor types. For this reason, these are the most studied integrins in cancer.[7]

In breast cancer, overexpression of $\alpha_v\beta_3$ integrin is associated with bone metastasis[18] and induces increased tumor growth and invasion in response to osteopontin.[19] $\alpha_v\beta_3$ expression is involved in the regulation of breast cancer cell response to chemotherapy and serves as a marker to chemosensitivity.[20] $\alpha_v\beta_3$ is upregulated in cells treated with microtubule interfering agents and not in cell lines that are resistant to these drugs; however, forced expression of the β_3 subunit increased cell resistance to paclitaxel.[21]

In glioblastoma, $\alpha_v\beta_3$ integrin is overexpressed at the invasive margins of the tumor and levels of fibronectin are increased, which is associated with enhanced cell motility and apoptosis resistance.[21]

In pancreatic tumor, the increased expression of $\alpha_v\beta_3$ integrin is associated with increased activation of MMP-2 and lymph node metastasis.[22]

In prostate carcinoma cell, $\alpha_v\beta_3$ integrin is expressed resulting in metastasis to bone because of an association between integrins and processes of attachment and migration involving laminin, fibronectin, and osteopontin.[21]

1.4. RGD Peptide and Peptidomimetics. The discovery of the structural basis of the recognition between integrins and their natural ligands together with the elucidation of the crystal structure of $\alpha_v\beta_3$ integrin and subsequent docking studies on this template have contributed to the rational design of a novel class of selective integrin inhibitors.[23]

The RGD sequence (Arg-Gly-Asp) (Figure 3A) was first discovered in the early 1970s by E. Ruoslahti as a cell attachment site in fibronectin.[24] Later, this sequence has been recognized as the minimal integrin sequence present in many natural ligands binding $\alpha_v\beta_3$ receptor as fibrinogen, fibronectin, vitronectin, plasminogen, thrombospondin, prothrombin, MMP-2, laminin, osteopontin, etc.[21] The RGD sequence is currently the basic module for a variety of molecules designed for the preferential binding to $\alpha_v\beta_3$ integrin and other integrins.[25] The affinity of RGD peptides for their ligands may be affected by steric conformation of the peptide.[26] Besides direct interactions between additional flanking groups and their receptor, the conformational features of the RGD motif can also be modulated. Indeed, the cyclization is commonly employed to improve the binding properties of RGD peptides, conferring rigidity to the structure (Figure 3B). In linear peptides, the fourth amino acid alters the binding specificity and the nature of residues flanking the RGD sequence could influence receptor affinity, receptor selectivity, and other biological properties.[26] Linear RGD peptide proved highly susceptible to chemical degradation. Since the rigidity conferred by cyclization prevents this, cyclic peptides are more stable, more potent, and more specific. In cyclic peptides, the RGD peptide sequence is flanked by other amino acids to build a ring system. These systems offer the possibility to present the RGD sequence in a specific conformation for a selected integrin.[26] Cyclization of a linear RGD pentapeptide including one of the amino acids (Phe) in the unnatural D-conformation (D-Phe) resulted in the cyclic peptide c(RGDfK) developed by Kessler and co-workers.[27] Another RGD peptide ligand, the so-called RGD4C, has also been studied as targeting ligand. Nevertheless, a disadvantage of this peptide is that this peptide can fold into different cyclic structures.[25] Structures, chemical modifications, affinity for $\alpha_v\beta_3$, and implications in targeted therapies of the different linear and cyclic RGD peptides have been reviewed by Temming et al.[25] Since the natural mode of interactions between integrin $\alpha_v\beta_3$ and RGD-containing proteins may involve multivalent binding sites, the idea to improve the integrin $\alpha_v\beta_3$ binding affinity with multivalent cyclic RGD peptides could provide more effective antagonists with better capability and higher cellular uptake through the integrin-dependent endocytosis pathway.[26]

Peptidomimetics (Figure 3E,F) are small protein-like chains designed to mimic a peptide and to target receptors with a higher affinity than the natural ligand. Because of the absence of peptidic linker, peptidomimetics offers advantages when compared to natural peptide such as increased physiological half-lives and oral bioavailability. Another important advantage of peptidomimetics is their ability to be amplified, enabling the screening of libraries to identify the most potent antagonists.[11]

2. RGD-BASED STRATEGIES FOR CANCER THERAPY

RGD-based strategies (Figure 4) include RGD antagonists, RGD conjugates, and RGD nanoparticles. This review aims to overview these strategies and particularly to highlight the position of RGD-based nanoparticles in cancer therapy and imaging.

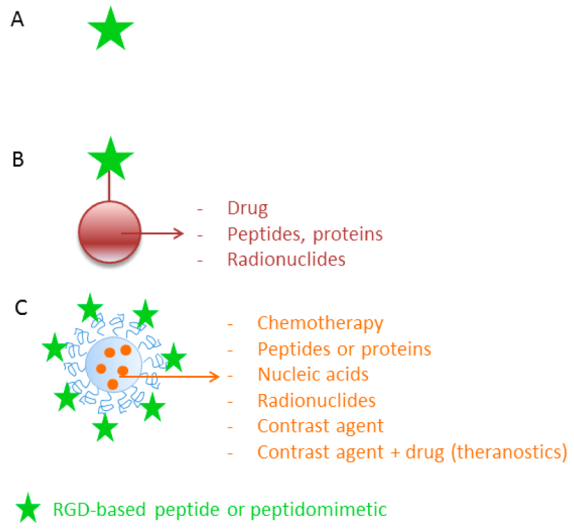

Figure 4. RGD-based strategies. (A) RGD antagonists. (B) RGD conjugates. The RGD-based peptide or peptidomimetic is conjugated to drugs or radionuclides with covalent links. (C) RGD peptides or peptidomimetics are grafted at the nanoparticle surface (polymeric nanoparticles, liposomes, polymeric micelles, etc.). These structures contains various agents such as anticancer drugs, peptides or proteins, nucleic acids, radionuclides, contrast agents, or a mixture of contrast agents and anticancer drugs (theranostics).

2.1. Antagonists (Non-Antibody) of $\alpha_v\beta_3$. Because of the expression of integrins in various cell types and their role in tumor angiogenesis and progression, integrins have become important therapeutic targets. Integrin antagonists currently preclinically studied or in clinical trials include (i) monoclonal

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Table 1. Nonexhaustive Examples of Recent Preclinical Studies of RGD-Targeted Nanocarriers Delivering Chemotherapeutics[a]

nanocarrier	therapeutic agent	targeting motif	tumor model	results	ref
nanoparticle	doxorubicin	cRGD	pancreatic/renal orthotopic mouse tumors	Metastases are suppressed by disrupting the associated vasculature.	47
nanoparticles	paclitaxel	GRGDS	TLT hepatocarcinoma	The targeting of RGD grafted nanoparticles to tumoral endothelium was more effective than nontargeted nanoparticles.	48,49
albumin nanoparticles	gemcitabine	RGD	BxPC3 pancreatic cancer cells	The uptake of RGD nanoparticles was found to be higher than nontargeted ones. The binding was mediated to $\alpha_v\beta_3$ receptor.	50
micelles	paclitaxel	c(RGDfK)	U87MG glioblastoma	The antiglioblastoma effect of targeted micelles was significantly longer than with other treatments.	51
HPMA conjugates	geldanamycin	c(RGDfK)	DU145 prostate tumor	Tumor accumulation was increased as compared to the conjugate without RGDfK.	52
liposomes	paclitaxel	cRGD	A549 lung adenocarcinoma	Targeted liposomes resulted in a lower tumor microvessel density than control.	53

[a]Examples were chosen to illustrate the variety of nanocarriers and targeting motifs.

antibodies (such as etaracizumab, Abegrin), (ii) RGD-based antagonists (peptidic or peptidomimetic), (iii) non-RGD antagonists (such as ATN-161, a non-RGD-based peptide inhibitor of integrin $\alpha_5\beta_1$), and (iv) integrin-targeted therapeutics. Monoclonal antibodies or non-RGD antagonists as anti-integrin therapies have been reviewed by Avraamides et al. and Sheldrake and Patterson, respectively.[8,21]

In preclinical studies, cilengitide effectively inhibited angiogenesis and the growth of orthopic glioblastoma.[7,30] A cyclic RGD peptide antagonist of $\alpha_v\beta_3$ and $\alpha_v\beta_5$, cilengitide (EMD 121974) showed favorable safety profiles and no-dose-limiting toxicities in phase I clinical trials.[31] Cilengitide is currently being tested in phase II trials in patients with lung and prostate cancer[32] and glioblastomas.[33,34] In addition, cilengitide has been shown to enhance radiotherapy efficiency in endothelial cell and non-small-cell lung cancer models.[35] Cilengitide in combination with temozolomide and radiotherapy administered in patients with newly diagnosed glioblastoma has also demonstrated promising efficacy.[36] Statement and opinion on clinical trials are discussed by Carter.[32] Several additional antagonists are studied preclinically but are not tested in clinical trials yet. The peptidomimetic S247 is an $\alpha_v\beta_3$ antagonist inhibiting breast cancer bone metastasis, decreasing colon cancer metastasis and angiogenesis, and increasing survival in mice.[37] The S137 peptidomimetic has also shown antimetastatic effects in xenograft tumor models.[38]

Paradoxically, Reynolds et al. found that the continuous infusion of very low concentrations of RGD-mimetic inhibitors stimulates tumor growth and angiogenesis by promoting VEGF-induced endothelial cell migration.[39] The enhanced tumor progression was the result of the increased tumor perfusion,[7] which could be exploited to increase the delivery of chemotherapeutic agents. Indeed antiangiogenic agents, such as cilengitide, have been shown to be more effective when used in combination with chemotherapy (e.g., gemcitabine).[40] As mentioned by Desgrosellier and Cheresh, it is important to note that antiangiogenic therapies (such as cilengitide) might target multiple cell types in the tumor microenvironment, including the tumor cells themselves, and therefore their antitumor effects may not be entirely due to antiangiogenic activity.[7] Until now, with regard to preclinical and clinical studies, the efficacy of $\alpha_v\beta_3$ antagonists, as antiangiogenic cancer treatments, still remains unclear. β_3 and β_5 knockout mice have shown that integrin inhibition may not be sufficient to completely block tumor angiogenesis.[41]

Currently ongoing clinical trials will allow clarifications about the therapeutic potential of these antagonists, which have shown safety profiles and no side effects in humans.[32] In the future, it will be necessary to develop not exclusively $\alpha_v\beta_3$ antagonists but dual antagonists of $\alpha_v\beta_3$ and $\alpha_v\beta_5$, since angiogenesis was shown to not be controlled by $\alpha_v\beta_3$ integrins alone.[11] In addition, $\alpha_v\beta_3$ antagonists provide the opportunity to address chemo- and radiotherapeutic agents to tumor endothelium.

2.2. RGD-Targeted Delivery of Therapy. Nanocarriers like liposomes, nanoparticles, micelles, etc. can be grafted at their surface with a targeting ligand such as an RGD-based sequence (Figure 4C). Several advantages are attributable to these nanocarriers: (i) the size of these nanocarriers (20−400 nm) leads to the "passive targeting" of tumors via the so-called enhanced permeability and retention (EPR) effect;[42] (ii) because of the size of these systems, renal filtration is avoided, leading to prolonged blood circulation times and longer accessibility of the ligand to target receptors within the tissue;[43] (iii) RGD-targeted nanocarriers may specifically address drugs to angiogenic endothelial cells and/or cancer cells by the binding of the RGD peptide to $\alpha_v\beta_3$ overexpressed by these cells, allowing the "active targeting" of the tumors;[44] (iv) RGD-targeted nanocarriers can be internalized via receptor-mediated endocytosis, which is not possible with single peptide constructs or with nontargeted nanocarriers; this is particularly interesting for the intracellular delivery of drugs to cancer cells.[25]

RGD-targeted nanocarriers have recently proven advantageous in delivering chemotherapeutics, peptides and proteins, nucleic acids, and irradiation.

a. Chemotherapy. The rationale behind the design of RGD-targeted nanocarriers is the delivery of various pharmacological agents to the $\alpha_v\beta_3$-expressing tumor vasculature. The cytotoxic drug destroys the tumor vasculature, resulting in the indirect killing of tumor cells induced by the lack of oxygen and nutrients. The tumor growth might be inhibited by preventing tumors from recruiting new blood vessels as suggested by Judah Folkman.[1] $\alpha_v\beta_3$ integrin is upregulated in angiogenic endothelial cells but also in several tumor cells, leading RGD-targeted nanocarriers to a potential double targeting. However, this double targeting is not yet exploited by systems delivering chemotherapeutics while it is described for integrin antagonists as etaracizumab or for RGD peptides.[45,46]

Examples of different RGD-targeted nanocarriers are displayed in Table 1. It is important to note that the first

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Table 2. Nonexhaustive Examples of Recent Preclinical Studies of Anticancer Proteins or Peptides Coupled to the RGD Peptide[a]

therapeutic agent	targeting motif	tumor model	results	ref
TNF-α	RGD4C	RMA lymphoma cells	This conjugate induces antitumor effects when combined with melphalan, improving its antineoplasic activity.	54
tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)	RGD4C	COLO-205 colorectal cancer and HT-29 colon cancer cells	The tumor growth was decreased.	55
endostatin	RGD	MGC-803 gastric cancer cells, SMMC-7721 hepatoma cells	Endostatin had no effects on tumor growth in vivo. However, with the peptide RGD, the conjugate showed significant antitumor results in vivo.	56
IL-12	RGD4C	NXS2 neuroblastoma	Due to RGD, localization of IL-12 to neovasculature significantly enhanced its antiangiogenic and its antitumor effect and decreased toxicity of IL-12.	57

[a]Examples were chosen to illustrate the variety of nanocarriers.

Table 3. Nonexhaustive Examples of Recent RGD-Targeted Anticancer Nucleic Acids[a]

nanocarriers	therapeutic agent	targeting motif	tumor model	results	ref
PEGylated PEI nanoparticles	siVEGFR2	RGD	N2A neuroblastoma	Tumor angiogenesis and tumor growth were inhibited with a marked loss of peritumoral vascularization.	59
conjugates	anionic antisense oligonucleotide (623)	RGDc	A375SM melanoma	The conjugate entered in cells via $\alpha_v\beta_3$ mediated endocytosis and produced significant increases in luciferase expression.	63
chitosan nanoparticles	siPLXDC1	RGDc	A2780 ovarian cancer cells	Tumor growth was inhibited compared with controls.	60
cationic liposomes	siP-gp or doxorubicin	RGD	MCF7/A breast adenocarcinoma	Tumor growth was inhibited when compared to nontargeted formulations and accumulation of siRNA or doxorubicin in tumors.	62
pH sensitive nanoparticles	anti-HIF-1α siRNA	RGDc	U87 glioma	Tumor growth was inhibited.	65
HK peptides	Luc siRNA	PEG-RGDc	MDA-MB-435 cells	Targeted nanoplexes administered intravenously were more effective than nontargeted ones in silencing luciferase.	64
PEGylated liposomes	anti-miR-296 AMO	RGDc	HUVEC (endothelial cells)	In vivo assessment of angiogenesis using Matrigel plug assay demonstrated that cRGD nanoparticles have potential for antiangiogenesis in miRNA therapeutics.	66

[a]Examples were chosen to illustrate the variety of nanocarriers.

results to show the inhibition of metastases with this kind of strategy were obtained with targeted nanoparticles loaded with doxorubicin.[47] The preferential activity of these nanoparticles on metastases suggests that growing metastatic tumors may have a greater dependence on angiogenic vessels.[7] PLGA-based nanoparticles grafted with the RGD peptide have been designed for the delivery of paclitaxel. The targeting to the tumoral endothelium was demonstrated both in vitro and in vivo. Moreover, therapeutic efficacy has been demonstrated by effective retardation of TLT tumor growth and prolonged survival times of mice treated by paclitaxel-loaded RGD nanoparticles when compared to nontargeted nanoparticles.[48,49]

b. Peptides and Proteins. Therapeutic proteins or peptides are becoming available for the treatment of cancer. However, several limitations exist including poor pharmacokinetics and side effects. Some proteins, such as cytokines or TNF-α, show potent anticancer activity. Cell-specific targeting of proteins could enhance the antitumor activity while reducing the systemic side effects. Some examples of anticancer peptides or proteins coupled to the RGD peptide are shown in Table 2.

c. Nucleic Acids. Because of their great potential as therapeutics in the field of oncology, vectorization of nucleic acids has become a very productive area of research. Introduction of nucleic acids in tumor cells can either promote gene expression by bringing a gene that is not expressed or is underexpressed into cells, or silence expression of specific genes, such as oncogenes. These effects are mediated by DNA or RNAi mediators, respectively. In addition to their need to be carried through the blood flow to their site of activity aiming to avoid (i) opsonization and elimination by the RES, (ii) clearance, (iii) degradation by nucleases, and (iv) interaction with plasmatic proteins, nucleic acids should also be targeted to a specific organ, tissue, or type of cells.[58] For these reasons, targeted nanosystems were designed. The specific and active targeting would diminish undesirable side effects, and reduce the necessary dose to observe an antitumor effect. In this perspective, the use of RGD peptide as a ligand to target angiogenesis-activated endothelial cells and tumoral cells via its binding to $\alpha_v\beta_3$ integrins is relevant.

The use of targeted nanosystems for gene therapy is a relatively new approach[58] (Table 3). DNA was complexed to PEI, a cationic polymer, to form polyplexes. When PEI was grafted with RGD moieties, VEGFR2 transfection into cells was enhanced.[59] Chitosan and poly-L-lysine were also described in the literature recently as promising nucleic acid delivery systems.[60,61] Other types of nanocarriers have been studied, such as targeted liposomes, targeted dendrimers, or direct conjugation of RGD moieties on nucleic acid molecules.[62,63] They all showed evidence of enhancement of gene transfer in vitro and/or in vivo.[64] It was demonstrated that addition of RGD moieties leads in cancerous cells to receptor-mediated endocytosis, explaining partly the increase in transfection.[58]

In addition to being efficiently internalized by targeted cells, nucleic acids, either DNA or siRNA, were also therapeutically efficient, showing that they were delivered, still active, to their subcellular site of action, respectively the nucleus and the cytoplasm. Delivery of biotherapeutics through $\alpha_v\beta_3$ integrin

targeting showed enhancement in both antitumor effects and antiangiogenic effects according to the targeted gene. These results bring also evidence that RGD-targeted nanocarriers do interact with tumoral endothelium tissue but also with tumor tissue itself.[64]

Even though an increasing number of nanocarriers are used for the intracellular delivery of nucleic acid, studies still demonstrated significant toxicity such as cell contraction, mitotic inhibition, formation of aggregates in blood, and a tendency to induce inflammatory response. Efforts have been done to reduce this toxicity. But their transfection efficiency needs further improvement for in vivo application.[58]

d. Radionuclides. Integrin antagonism may also benefit radiotherapy. Exposure of tumors to irradiation causes transient upregulation of $\alpha_v\beta_3$ on tumor blood vessel endothelium.[35,67] This upregulation may be used as a method of drug delivery or to enhance the effects of radiotherapy.[21] The $\alpha_v\beta_3$ antagonist cilengitide has been shown to increase cell sensitivity to radiation in proportion to the level of integrin expression.[35]

In general, an integrin $\alpha_v\beta_3$ targeted radiopharmaceutical can be divided into three parts: the targeting biomolecule (e.g., cyclic RGD peptide), a linker, and a radiometal chelate. RGD-based targeted agents are designed for either diagnosis imaging (see section 3.1) or radionuclide therapy. Several clinically relevant radionuclides have been used for labeling bioactive peptides either for diagnostic imaging (^{99m}Tc, ^{111}In, $^{66/68}$Ga, ^{18}F, ^{123}I, ^{64}Cu) or for therapy (^{111}In, $^{64/67}$Cu, ^{90}Y, ^{177}Lu, ^{213}Bi). Radiopharmaceuticals can be used for identification of receptor-positive tumor lesions, treatment planning, and dosimetry. When labeled with a therapeutic radionuclide, the same peptide can be utilized for targeted radionuclide therapy. In this case, radiopharmaceuticals bind specifically to target receptors on tumor cells and deliver an effective radiation dose to tumor cells with minimal damage to normal tissues.[68] Rajopaddhye and co-workers were the first to use cyclic RGD dimers as targeting biomolecules for the development of therapeutic (^{90}Y and ^{177}Lu) radiotracers.[26] The RGD peptide DOTA-E-[c(RGDfK)]2 was labeled with with ^{90}Y for therapy experiments. The therapeutic efficacy of 37 MBq ^{90}Y-DOTA-E-[c(RGDfK)]2 was compared with that of 37 MBq administered in five equal portions. No difference in tumor growth between the fractionated and the nonfractionated therapy was observed.[69] Another example is the HPMA–RGD conjugates containing side chains for ^{99m}Tc (imaging) and ^{90}Y (therapeutic). Treatment of in vivo DU145 xenografts showed significant decreases in tumor volume for 250 μCi ^{90}Y doses when compared to control. HPMA–RGD conjugates inhibited $\alpha_v\beta_3$ mediated endothelial adhesion and remained active while HPMA homopolymer showed no activity.[70] Recently, RGD conjugate gold nanorods were designed to induce the radiosensitization in melanoma cancer cells by downregulating $\alpha_v\beta_3$ expression in addition to induction of a higher proportion of cells within the G_2/M phase.[50]

e. Theranostics. Antibodies, liposomes, nanoparticles, and micelles carrying contrast agents have already shown potential to detect diseases and visualize various important aspects for the drug delivery process. In addition, nanomedicines are being prepared to combine diagnostic and therapeutic agents. Applications of "nanotheranostics" are extended: nanotheranostics allow (i) the noninvasive evaluation of the biodistribution and the target site accumulation of nanoparticles; (ii) the monitoring and the quantification of the drug release; (iii) the facilitation of therapeutic intervention relying on triggered drug release; (iv) the prediction of therapeutic response; and (v) the longitudinal monitoring of the efficacy of therapeutic interventions.[71] By the noninvasive assessment of the biodistribution and the target site distribution, nanotheranostics allow the optimization of drug delivery systems in order to improve the treatment of individual patients and to better understand several important aspects of drug targeting to pathological sites.[72] Hence, Lee and colleagues developed "all-in-one" magnetic nanoparticles. These nanoparticles contained iron oxides as contrast agent, siRNA as therapeutic agent, an RGD-containing peptide as target ligands of the $\alpha_v\beta_3$ integrins, and a fluorescent dye for fluorescent microscopy.[71] Other RGD-targeted nanotheranostics were studied such as HPMA–drug conjugate containing DY-615 as contrast agent[73] or PLA micelles containing SPIO as contrast agent.[74] Interestingly, RGD-targeted paramagnetic nanoparticles were designed for both delivering antiangiogenic therapy and detecting the tumor response. In this study, it was demonstrated that the characterization of angiogenesis with MR molecular imaging may identify tumors with low levels of neovasculature that may respond poorly to antiangiogenic therapy.[75]

f. The Case of iRGD Cyclic Penetrating Peptide. Interestingly, two recent papers[76,77] have shown that the iRGD cyclic peptide has the potential to selectively deliver a large variety of therapeutic or diagnostic agents to a tumor site. The cyclic iRGD is constituted from CRGDK/RGPD, containing a cryptic CendR motif, CRGDK/R that possesses CendR-like tissue and cell penetrating activities.

Intravenously injected compounds coupled to iRGD bound to tumor vessels and spread into the extravascular tumor parenchyma, whereas conventional RGD peptides (CRGDC and RGD-4C) only delivered the cargo to the blood vessels. In a first step, the intact peptide binds to the endothelial cell expressing α_v integrins. In a second step, a protease cleaves iRGD and exposes the cryptic CendR motif which can interact with the neuropilin-1 receptor and thereby increase tumor vascular permeability (Figure 5). The proteolytically processed

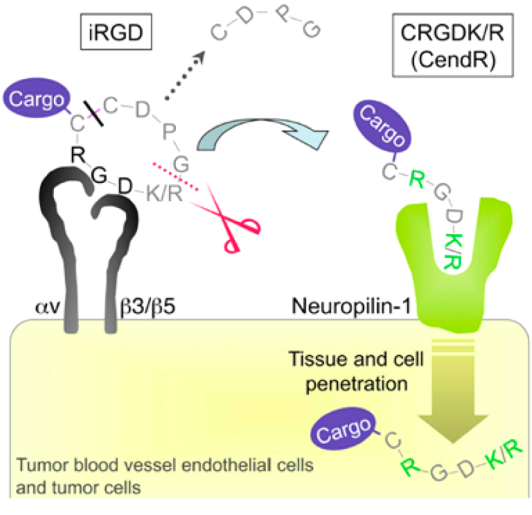

Figure 5. Tissue penetrating iRGD. Penetration mechanism of iRGD. The iRGD peptide binds to α_v integrin expressed by tumor blood vessel endothelial cells and tumor cells. After the binding, the peptide is cleaved by proteases to expose the cryptic CendR element. This CendR element binds to neuropilin-1 and penetrates into cells and tissue. The peptide can also penetrate into tumors while carrying a cargo attached to the N-terminus of the iRGD peptide.[76]

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CRGDK fragment has lost most of its affinity to the integrins. Instead, the CRGDK fragment acquires an affinity for neuropilin-1 that is stronger than its residual affinity for α_v integrin. These changes likely facilitate the transfer of CRGDK from integrins to neuropilin-1, and the resulting penetration activities. Studies on the time dependence of iRGD and penetration further supported the importance of α_v integrin and neuropilin-1 expression in this process. M21 human melanoma cells expressing both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ bound iRGD phage, whereas variants lacking expression of these integrins did not, confirming the α_v integrin dependency of iRGD binding. Importantly, tumor cells strongly positive for neuropilin-1 were particularly effective at accumulating and retaining iRGD.

The potential of this tissue-penetrating peptide was demonstrated for preclinical applications by performing MRI and tumor treatment studies.[76] Remarkably, this penetrating peptide works not only when conjugated to the payload but also when it is coadministered. Systemic injection with iRGD improved the therapeutic index of drugs of various compositions including small molecules (doxorubicin), nanoparticles (nab-paclitaxel (Abraxane) and doxorubicin liposomes) and a monoclonal antibody (trastuzumab). Thus, coadministration of iRGD may be a valuable way to enhance the efficacy of anticancer drugs while reducing their side effects, a primary goal of cancer therapy research.[77]

3. RGD-BASED STRATEGIES FOR CANCER DIAGNOSIS

Molecular imaging for noninvasive assessment of angiogenesis represents potential interest for clinicians since antiangiogenic drugs have been successfully used in cancer patients. Traditionally, cancer treatments with classical chemotherapies are used with morphological imaging to provide indices of therapeutic response. The most common imaging techniques are positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and different optical imaging techniques with high sensitivity. Great interest has emerged in identifying and developing biomarkers of early tumor response. One promising approach is to identify molecular markers of angiogenesis by conjugating a specific ligand recognizing overexpressed receptors in angiogenic tumors to imaging probes. In this area, one of the most promising and best studied targets is the integrin $\alpha_v\beta_3$.[78] Even though optical imaging still cannot compete with PET, CT, or MRI in clinical applications, the advantages of optical imaging are (i) its convenient use, (ii) its sensitivity, (iii) its cost-effectiveness, and (iv) its nonionization safety.[79]

3.1. Radionuclide. The potential of RGD-containing peptides to serve as vehicles for targeting tumors with radionuclides has been investigated by several groups. It was found that the cyclic pentapeptide *cyclo*(Arg-Gly-Asp-D-Phe-Val) was a 100-fold better inhibitor of cell adhesion to fibronectin compared to the linear peptide. Moreover, a hydrophobic amino acid in position 4 increased its affinity for $\alpha_v\beta_3$. Based on these results, Haubner et al. designed five peptides that could be radioiodinated by introducing a tyrosine residue. Two peptides were studied in vivo highlighting a rapid clearance from blood.[80] Therefore, RGD peptides were improved by conjugating them with sugar amino acids. [^{18}F]Galacto-RGD was designed and was the first radiotracer developed for PET and SPECT and used in patients.[80] Imaging studies clearly showed that the accumulation of [^{18}F]galacto-RGD correlated well with the tumor $\alpha_v\beta_3$ expression levels in cancer patients.[81] Based on these results in patients, many integrin $\alpha_v\beta_3$-targeted optical imaging agents have been built on the cyclic RGD template similarly. Two Cy.5-labeled cyclic RGD analogues (Cy5.5-(RGDfK) and Cy5.5-(RGDyK)) were first reported for in vivo optical imaging of integrin $\alpha_v\beta_3$ positive tumors in mouse models and showed high contrast images.[82,83] Additionally, many conjugates are designed for dual optical and nuclear imaging. For example, ^{111}In-DTPA-Lys(IRDye800)-c(KRGDf) was injected to mice and was imaged by both gamma scintigraphy and NIR fluorescence optical camera. These two techniques enabled noninvasive detection of the probe bound to integrin $\alpha_v\beta_3$-positive tumors. Optical images provided improved resolution and sensitive detection of the superficial lesions while gamma images provided sensitive detection of deeper structures.[84]

The second radiotracer that was used in patients was ^{99m}TC-labeled RGD-containing peptide (NC100692). This SPECT tracer was introduced recently for imaging $\alpha_v\beta_3$ expression in patients with breast cancer.[85] The data in subjects with suspected breast lesions suggest that ^{99m}Tc-NC100692 scintigraphy may be effective in detecting malignant lesions. To improve the efficiency of tumor targeting and to obtain better in vivo imaging, multimeric RGD peptides were synthesized and evaluated. Multimers of RGD peptides have been labeled with [^{18}F] for PET imaging using polyethyleneglycol (PEG) amino acid or other spacers to improve pharmacokinetics and tumor targeting efficacy. Although the potential benefit of multivalent probes is generally accepted, the exact mechanism of the enhanced accumulation in $\alpha_v\beta_3$-expressing tumors is unclear.[86] The concept of multimerization has also been successfully investigated with other RGD-ligand systems such as ^{64}Cu, ^{99m}Tc, and ^{68}Ga-labeled peptide dimers, tetramers, and even octamers. Their high tumor uptake and fast renal excretion make the ^{99m}Tc-RGD system a promising radiotracer for noninvasive imaging of the integrin $\alpha_v\beta_3$-positive tumors by SPECT.[26,87]

The third radiotracer currently in clinical trial is [^{18}F]-AH111585 also called [^{18}F]fluciclatide, which is a novel cyclic RGD-based radioligand. The original peptide sequence (RGD-4C) was optimized to improve the in vivo stability and to increase the plasma half-life. This radioligand had successfully imaged metastatic breast cancer lesions: all tumors of 7 patients were visible with [^{18}F]fluciclatide PET and compared with images obtained by computer tomography. Similar work has also been published using another radiolabeled RGD agent in malignant melanoma.[88,89]

3.2. Nanoparticle-Based Approach. Targeted nanoparticles for molecular imaging present advantages over conventional imaging strategies: (i) thousands or even more imaging labels can be attached to a single nanoparticle increasing the signal intensity; (ii) nanoparticles can be functionalized as multimodality imaging systems by using different imaging labels; (iii) due to their size, they preferentially access tumor cells through fenestrations. However, the prolonged blood half-life of nanoparticles does not allow imaging at early time points after injection.[78]

[^{111}In]-Labeled perfluorocarbon nanoparticles[90] or single-walled carbon nanotubes (SWNT)[87] have been studied for PET imaging. Targeted nanocarriers have also been developed for MRI. The first approach for imaging $\alpha_v\beta_3$ expression was Gd^{3+}-containing paramagnetic liposomes. Unfortunately, the MR signal highlighted that nanoparticles penetrated into the leaky vasculature but did not migrate into the interstitium

substantially.[91] Nevertheless, the same group developed other paramagnetic nanoparticles able to image very small regions of about 30 mm^3 of angiogenesis associated with melanoma tumors xenografts.[92]

Gd^{3+} for enhancing the T1 contrast ("positive contrast") can only be reliably detected at millimolar levels. Superparamagnetic iron oxide nanoparticles (SPION) can be detected at a much lower concentration because of the high susceptibility induced by these particles leading to a decrease of the signal in T2 and especially T2* weighted sequences ("negative contrast").[78] In vivo studies involving $\alpha_v\beta_3$-targeted SPION have shown a strong specific uptake into tumors compared to untargeted nanoparticles, indicating the potential for these peptides for future clinical applications.[93−95] RGD-SPION were found to be internalized by $\alpha_v\beta_3$-expressing cells through receptor-mediated endocytosis in contrast to the phagocytic mechanism observed for particles without RGD ligands.[94] MR studies showed greater accumulation of RGD-SPION in the endothelial cells of tumor vasculature (significant changes of the R2 relaxation rate of tumors) than nontargeted SPION. The pattern of contrast enhancement detected on MR images was consistent with the known vasculature structure of the type of tumor studied. The preferential accumulation of RGD-SPION in endothelial versus tumor cells can be due to the larger size of nanoparticles decreasing their ability to diffuse out of the vasculature.[93,94,96]

The iRGD cyclic penetrating peptide (described previously) was also evaluated for preclinical applications, using MRI. Hence the iRGD was linked to superparamagnetic iron oxide nanoworms (80 nm long and 30 nm thick). Both the MRI results and optical imaging indicated that the iRGD was capable of delivering diagnostics to tumors and that the tumors were more efficiently visualized with this peptide than with conventional RGD peptides.[76]

Various types of nanoparticles such as quantum dots (QDs), carbon nanotubes, or gold nanoparticles have been developed for cancer imaging. Some new innovative multifunctional nanoparticles with improved targeting efficacy and biocompatibility for multimodality imaging and targeted therapy have been discovered by integrating integrin-targeting, optical imaging, and even complementary multimodality imaging motifs into nanoparticle constructs.[79] QD705-RGD was the first QD developed for targeting and imaging of integrin $\alpha_v\beta_3$-positive tumor vasculature.[97] The major perspective of nanoparticle-based optical agents consists of their ability to multiplex optical imaging with other imaging modalities and targeted therapy, serving as an attractive type of theranostics for simultaneous imaging and targeted therapy.[79]

4. DISCUSSION AND CONCLUSIONS

4.1. Is the Intracellular Distribution Altered Because of the RGD Peptide? The potential advantage of targeted delivery may result from an altered intracellular distribution. Both targeted and nontargeted systems arrive at the tumor via the EPR effect, after which the mechanism of tumor cell internalization is enhanced by the presence of surface ligands.[98,99] It was demonstrated that RGD-grafted nanoparticles enter cells through integrin-mediated endocytosis or clathrin-mediated endocytosis and are then localized in the perinuclear regions.[7,48,100,101] When nontargeted nanocarriers arrive at target cells, no binding occurs. The drug is gradually released from the nanocarrier and is taken up by the cell as free drug, using standard uptake mechanisms. On the contrary, for targeted nanocarriers, once the ligand binds to its receptor, nanocarriers are taken into the cell by receptor-mediated endocytosis. Assuming that the nanocarrier is stable in the environment of the endosome, the drug is gradually released within the cell. It is probably a major explanation of the success of targeted systems.[7,99]

4.2. Are Passive and Active Targetings Complementary? For nongrafted nanocarriers, only the EPR effect can explain the accumulation of drug-loaded nanocarriers into the tumor. But for RGD-grafted nanocarriers, what is really happening? The Figure 6 schematizes the different targeting

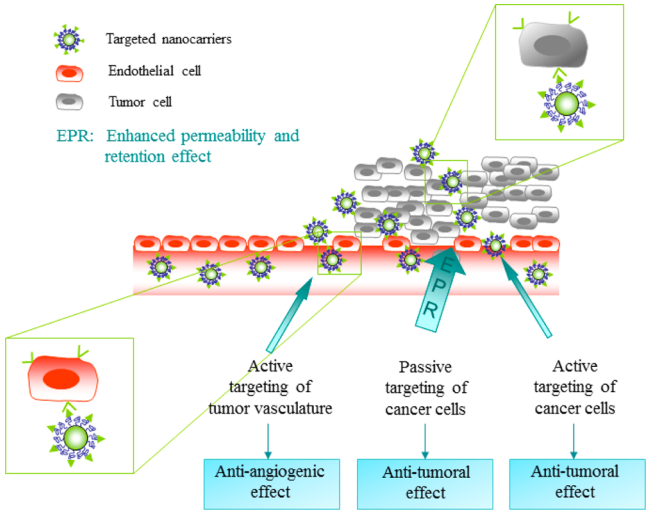

Figure 6. Schematic representation of targeting mechanisms of RGD-grafted nanocarriers.

mechanisms of RGD-grafted nanocarriers. $\alpha_v\beta_3$ integrin is expressed on endothelial cells. Circulating RGD nanocarriers can bind to these integrins. Nanocarriers are then internalized probably via receptor-mediated endocytosis.[99] Subsequently, intracellular drug cytosolic release occurs, followed by direct killing of endothelial cells. The destruction of the endothelium in solid tumors can result in the death of tumor cells induced by lack of oxygen and nutrients, a theory proposed by J. Folkman.[1] This is the antiangiogenic effect of the active targeting of drug-loaded RGD nanocarriers. RGD nanocarriers may also likely enter the tumor via the EPR effect. This passive targeting process facilitates tumor tissue binding, followed by cellular uptake (internalization). The intracellular drug release then leads to cancer cell killing. Since cancer cells are also known to express $\alpha_v\beta_3$ integrin, once penetrated into the tumor interstitial fluid through EPR effect, RGD nanocarriers may also bind to cancer cells.[7] Consequently, the nanocarrier intratumor distribution shifts from the extracellular compartment to the tumor cell intracellular compartment.[99] The EPR effect combined with the active targeting of cancer cells leads thus to the antitumoral effect of drug-loaded RGD nanocarriers. This double active targeting of both endothelial and cancer cells is not yet really exploited nor discussed in the literature. Only a few papers described the upregulation of $\alpha_v\beta_3$ integrin by tumor and tumor endothelial cells for a double targeting.[45,46] For example, lipid-based particles showed in vitro selectivity for $\alpha_v\beta_3$ expressing M21 tumor cells. When mice bearing M21 tumors (the $\alpha_v\beta_3$-negative variant of the M21 melanoma) were treated with a single intravenous dose of these nanoparticles, apoptosis of tumor endothelium was observed, as well as

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apoptosis among the tumor cells proximal to apoptotic vessels. The absence of $\alpha_v\beta_3$ on the tumor cells suggests that the latter effect was due to the vascular effect, rather than a direct effect on the tumor cells.[102] By contrast, many authors described a double targeting using one specific ligand addressed to endothelial cells and another to cancers cells. These systems are called "multifunctional nanocarriers".[103]

4.3. Is the RGD Peptide a Good Ligand to Target Tumors? The RGD peptide is an effective ligand for tumor targeting since it has been shown that integrin $\alpha_v\beta_3$ is overexpressed not only on tumoral endothelium but also on cancer cells, for a lot of cancer cell lines.[7,45] Nanocarriers grafted with the RGD peptide may thus be considered as a double targeting system. To our knowledge, other largely described ligands such as folate or transferrin are only expressed on cancer cells. Moreover, integrins $\alpha_v\beta_3$ are known to be poorly expressed in non-angiogenesis activated endothelial cells. On the contrary, transferrin is expressed at elevated levels on cancer cells but also on brain capillaries, endocrine pancreas, or Kupffer cells of the liver.[104] In addition, some ligands, such as folate, that are supplied by food, show naturally high concentrations in the human body and might compete with the nanoparticle-conjugated ligand for binding to the receptor, effectively reducing the intracellular concentration of delivered drug.[105] The major advantage of the RGD nanocarriers is precisely this possible double targeting. First, endothelial cells are targeted because of their expression of the integrin $\alpha_v\beta_3$, and cancer cells (which also expressed the integrin $\alpha_v\beta_3$) are also targeted after extravasation of nanocarriers. Second, the potential normalization resulting of antiangiogenic properties (RGD targeting and intrinsic property of some anticancer drugs) of nanocarriers could enhance the delivery of drugs into tumors. Furthermore, this is the rationale of many combinations of treatments tested in clinical trials. Indeed, in human, because of the slow growth of tumors (compared with tumor-bearing mice models), the maturation of tumor vasculature is more complete, and then antiangiogenic therapies combined with chemotherapy or radiotherapy are therefore a rational strategy for tumor eradication.[106]

In conclusion, one of the major pitfalls in the field of tumor-targeted drug delivery relates to the fact that the EPR is often misinterpreted. The EPR effect is a highly heterogeneous phenomenon, which varies substantially from tumor model to tumor model, as well as from patient to patient. Another aspect relates to the overestimation of the potential usefulness of active drug targeting. Theorically, the benefit of targeted nanoparticles is to be retained more efficiently and more rapidly than nontargeted ones. However, the introduction of targeting moieties often leads to an increase in immunogenicity and in protein adsorption. The main advantage of actively targeted nanoparticles over passively targeted formulations is that they are taken up by cancer cells more efficiently.[46]

The approach of using RGD peptides and peptidomimetics, either in the targeted nanomedicine field or as radiopharmaceuticals, has successfully been translated from preclinical studies to bedside, meaning that more progress is to be expected. Because of a large number of clinical trials performed to date and several approved formulations combined with other treatment modalities (such as standard chemotherapy or radiotherapy), it can be predicted that, in years to come, tumor-targeted strategies including nanomedicines will be integrated in combined modality anticancer therapy. Therefore, efforts should focus on designing ever more carrier material, on a better understanding of biological principles, but also on establishing rational combination regimens. Moreover successful clinical data on tumor imaging indicate that the concept of personalized medicine could be a good tool to overcome major pitfalls in tumor-targeted drug delivery. Thus in the next step, large-scale trials using radiopharmaceuticals within the context of response assessment or evaluation of patient prognosis are needed to define the ultimate role of imaging of integrin expression in the clinic.[27]

AUTHOR INFORMATION

Corresponding Author

*Université catholique de Louvain, Pharmaceutics and Drug Delivery, Avenue Mounier, 73 B1 73 12, B-1200 Brussels, Belgium. Phone: +32 2 7647320. Fax: +32 2 7647398. E-mail: veronique.preat@uclouvain.be.

Notes

The authors declare no competing financial interest.

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