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## Integrin $\alpha_v\beta_3$ -Targeted Cancer Therapy

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### Abstract

Anti-angiogenesis is a promising strategy for the treatment of cancer. Integrins, consisting of two noncovalently bound transmembrane  $\alpha$  and  $\beta$  subunits, are an important molecular family involved in tumor angiogenesis. The blockade of integrin signaling has been demonstrated to be efficient to inhibit tumor growth, angiogenesis, and metastasis. Among all the integrins,  $\alpha_v\beta_3$  seems to be the most important one during tumor angiogenesis. The inhibition of integrin  $\alpha_v\beta_3$  signaling with antibodies, peptides, peptidomimetics, and other antagonists has great potential in the treatment of cancer. In addition, integrin  $\alpha_v\beta_3$  is highly expressed on activated endothelial cells, new-born vessels as well as some tumor cells, but is not present in resting endothelial cells and most normal organ systems, making it a suitable target for anti-angiogenic therapy. In this article we will review the role of integrin  $\alpha_v\beta_3$  in angiogenesis, present recent progress in the use of integrin  $\alpha_v\beta_3$  antagonists and integrin-targeted delivery systems as potential cancer therapeutics, and discuss future perspectives.

### Keywords

integrin  $\alpha_v\beta_3$ ; angiogenesis; metastasis; anti-angiogenic therapy

## INTRODUCTION

Despite positive initial responses to therapeutic drugs, many tumors become refractory to cytotoxic agents, leading to the failure of cancer treatment. Two major reasons have been found to be responsible for the therapeutic failures. First, the physiological barriers within the tumor impedes delivery of therapeutic agents at an effective concentration to tumor cells. Second, the drug resistance of the tumor reduces the effectiveness of available drugs [Jain, 2001]. To overcome or mitigate the problems, Folkman [1971] first introduced the concept that inhibition of angiogenesis (anti-angiogenesis) might be an effective strategy to treat human cancers. The intensive search for angiogenesis inducers and inhibitors has been ongoing ever since.

Tumor angiogenesis, or the sprouting of new vessels from preexisting vasculature, is well recognized as an essential mechanism for tumor growth and development of metastasis [Carmeliet and Jain, 2000; Folkman, 1995a, 2002]. Without the formation of neovasculature to provide oxygen and nutrients, tumors cannot grow beyond 1–2 mm in size [Folkman, 1995b; Sharma et al., 2001]. Once vascularized, previously dormant tumors begin to grow rapidly, invade surrounding tissues (invasion), and transfer to distant sites in the body

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(metastasis). The “angiogenic switch” depends on the balance between pro-angiogenic molecules such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF), and anti-angiogenic molecules (e.g., angiostatin, endostatin, and thrombospondin) [Cai and Chen, 2006; Carmeliet and Jain, 2000].

As cancer cells become efficiently invasive and metastatic, partial degradation of the extracellular matrix (ECM) at the invasion front is required. Integrins, a family of cell adhesion molecules, are involved in a wide range of cell–ECM and cell–cell interactions [Brooks et al., 1994; Folkman, 2002; Jin and Varner, 2004; Kumar, 2003]. Although endothelial cells express many different integrins,  $\alpha_v\beta_3$  appears to be the most important integrin for angiogenesis [Brooks et al., 1994; Kumar, 2003]. Integrin  $\alpha_v\beta_3$  is highly expressed on activated endothelial cells and new-born vessels, but is absent in resting endothelial cells and most normal organ systems, making it a suitable target for anti-angiogenic cancer therapy. In addition, it is also expressed on some tumor cells, allowing for both tumor cell and tumor vasculature-targeted therapy. To date, numerous anti-angiogenic therapies based on integrin  $\alpha_v\beta_3$  antagonism, including antibodies, peptides, small molecules, small interfering RNA (siRNA), combination therapy, and targeted delivery of anti-cancer agents, have been investigated. Our review will focus on the integrin  $\alpha_v\beta_3$ -targeted therapies of cancers, and address the most recent development.

## INTEGRIN STRUCTURE AND SIGNALING

Integrins represent a subclass of cell adhesion molecules connecting the cytoskeleton with the extra-cellular matrix (ECM) or other cells. They consist of two genetically nonrelated subunits,  $\alpha$  and  $\beta$ , which are noncovalently associated with each other. In mammals, there are 18  $\alpha$  and 8  $\beta$  subunits capable of assembling at least 24 different functional heterodimers [Cai and Chen, 2006; Hynes, 2002] (Fig. 1). The alternative splicing of mRNA of some  $\alpha$ - and  $\beta$ -subunits and posttranslational modifications of integrin subunits adds further diversity to the integrin family. Each individual integrin subunit consists of a large extra-cellular domain (~1,000 and ~750 residues), a single transmembrane domain, and a short cytoplasmic tail (~20 and ~50 residues, except for  $\beta_4$ ) [Alghisi and Raegg, 2006; Hood and Cheresh, 2002]. The assembled integrin heterodimer binds a specific set of endogenous ligands, including ligands in the ECM, soluble ligands, and ligands on other cell surfaces [Eble and Haier, 2006]. Upon ligand binding, the cytoplasmic tail contacts cytoskeletal filaments and proteins to initiate a signaling cascade, including a series of intracellular signaling events (both mechanical and chemical signals) that start from the recruitment and activation of Src kinases via phosphorylation of focal adhesion kinase (FAK) [Guo and Giancotti, 2004]. After signaling, the integrins play their roles not only in adhesion to ECM ligands or counter-receptors on adjacent cells, but also by initiating signaling that induces cell spreading, migration, survival, proliferation, and differentiation [Schwartz, 2001].

## INTEGRIN $\alpha_v\beta_3$ IN TUMOR ANGIOGENESIS

The integrin  $\alpha_v\beta_3$ , also known as the vitronectin receptor, consists of a 125-kDa  $\alpha_v$  subunit and a 105-kDa  $\beta_3$  subunit. Integrin  $\alpha_v\beta_3$  binds a wide range of ECM molecules with an Arg-Gly-Asp (RGD) triple-peptide motif, including fibronectin, fibrinogen, von Willebrand factor, vitronectin, and proteolysed forms of collagen and laminin, whereas other integrins such as  $\alpha_5\beta_1$  can only selectively bind fibronectin [Hood and Cheresh, 2002; Hsu et al., 2007; Van der Flier and Sonnenberg, 2001]. Integrin  $\alpha_v\beta_3$  has diverse roles in several distinct processes, such as osteoclast-mediated bone resorption, angiogenesis, and pathological neovascularization, and tumor metastasis [Wilder, 2002]. For angiogenesis,  $\alpha_v\beta_3$  appears to be the most important one among all the integrins [Brooks et al., 1994; Kumar, 2003]. In addition to interacting with a number of ECM proteins, integrin  $\alpha_v\beta_3$  has

been shown to be associated with fibroblast growth factor-2 (FGF2), metalloproteinase MMP-2, activated PDGF, insulin, and VEGF receptors, facilitating the optimal activation of cell proliferation, invasion and preventing apoptosis [Kumar, 2003]. More importantly, the inhibition of  $\alpha_v\beta_3$  integrin activity by monoclonal antibodies (mAbs), cyclic RGD peptide antagonists, and peptidomimetics has been shown to induce endothelial cell apoptosis [Meerovitch et al., 2003] and to inhibit angiogenesis [Kumar, 2003].

The crucial roles of integrin  $\alpha_v\beta_3$  in tumor angiogenesis have led to a promising strategy to block its signaling by antagonists, as this would theoretically inhibit the tumor angiogenesis or enhance the efficacy of other tumor therapeutics. In addition, the high expression of integrin  $\alpha_v\beta_3$  on tumor new-blood vessels and some tumor cells makes the integrin  $\alpha_v\beta_3$  a suitable marker for cancer-targeted delivery of cytotoxic therapeutics (Fig. 2).

## INTEGRIN $\alpha_v\beta_3$ INHIBITORS

### Antibodies

**LM609/Avastin**—LM609 (Avastin, bevacizumab, Genentech) is a mouse anti-human integrin  $\alpha_v\beta_3$  mAb, which cross-reacts with  $\alpha_v\beta_3$  originated from rabbits, chicken, and hamsters but not from rodents [Cheresh, 1987]. The primary anti-angiogenic mechanism of LM609 was found to be its blocking of bFGF and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) induced angiogenesis [Friedlander et al., 1995]. Compared with other mAbs, which recognizes either the  $\alpha_v$  or  $\beta_3$ , LM609 is more specific and effective. Intravenous (i.v.) injection of LM609 was reportedly capable of effectively reducing tumor growth and angiogenesis in a severe combined immunodeficient (SCID) mouse/human chimeric model [Brooks et al., 1995].

Based on the positive preclinical results from LM609, several humanized versions of the mAb were developed to reduce the possible HAMA (human anti-mouse antibody) reaction of LM609 and optimize its serum half-life. The humanized versions of LM609 retained the antigen and epitope specificity of the original murine mAb, but not the cross-reaction with integrin  $\alpha_{IIb}\beta_3$  or  $\alpha_v\beta_5$ . The binding affinity of the humanized antibodies was also improved after optimization of the complementarity determining regions (CDRs) LCDR1, HCDR1, and HCDR3. After conversion into human IgG1 using a mammalian expression vector, the humanized mAb Vitaxin I (MEDI-523) showed significant inhibition of tumor growth in a Kaposi's sarcoma mouse model and partially blocked the binding of human immunodeficiency virus (HIV-1) Tat protein to integrin  $\alpha_v\beta_3$  [Rader et al., 2002]. In the subsequent Phase I clinical trial [Gutheil et al., 2000], Vitaxin I has been proven safe for therapeutic use, however, it had limited efficacy in patients with advanced malignancies. The affinity of this antibody was further increased using stepwise maturation with phage expression libraries [Wu et al., 1998]. The resulting antibody was named Abegrin (Vitaxin II, MEDI-522), which was later licensed to MedImmune, Inc. (Gaithersburg, MD). In a phase I study [Patel et al., 2001], 15 patients received Abegrin for a period of 6 months. Although the toxicity profile of Abegrin was quite satisfactory, it failed to show evidence of antitumor activity. In another clinical trial [Posey et al., 2001], patients with metastatic cancer who failed standard therapy received intravenous doses of 10, 50, or 200 mg in cohorts of three patients; again, no significant toxicity or immune responses were observed in the three different dose levels, nor were any anti-tumor responses noted. One potential limitation of Abegrin is that it blocks only  $\alpha_v\beta_3$ -mediated angiogenesis, not the angiogenic pathways of other integrin receptors. Other  $\alpha_v$  integrins, such as  $\alpha_v\beta_5$ , can also mediate tumor angiogenesis, and there is evidence that  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  promote angiogenesis via distinct pathways:  $\alpha_v\beta_3$  through bFGF and TNF- $\alpha$ , and  $\alpha_v\beta_5$  through VEGF and TGF- $\alpha$  [Friedlander et al., 1995]. Therefore, the combination of two or more angiogenic pathway inhibitors may be more effective than any single blocking agents alone for anti-angiogenic cancer therapy [Mullamitha et al., 2007].

**CNTO 95**—CNTO 95 (Centocor) is a fully humanized antibody that recognizes multiple  $\alpha_v$  integrins. It binds to purified human  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  with high affinity (~200 pM) and to  $\alpha_v$  integrin expressing human cells with a  $K_d$  of 1–24 nM [Trikha et al., 2004], but does not bind to murine angiogenic integrins. In vitro, CNTO 95 inhibited human melanoma cell adhesion, migration and invasion [Chen et al., 2008; Trikha et al., 2004]. In a rat aortic ring sprouting assay, CNTO 95 completely inhibited sprouting in a dose of ~70 nM. In an in vivo nude mouse model, CNTO 95 inhibited growth of human melanoma tumors by ~80% with a dose of 10 mg/kg (3 times/week) [Trikha et al., 2004]. In a phase I clinical trial [Mullamitha et al., 2007], CNTO 95 was generally safe and well tolerated. Among the 24 enrolled patients, six patients received extended therapy, including one patient with a prolonged response. More importantly, a partial response was observed in a patient whose tumor did not express  $\alpha_v\beta_3$  integrin but did express  $\alpha_v\beta_1$  integrin, which may be evidence that CNTO 95 could potentially be more effective for tumor therapy than the single-integrin inhibitors, due to its multiple integrin inhibition property.

**c7E3**—The c7E3 Fab (abciximab; ReoPro; [Centocor]) and m7E3 (Fab')<sub>2</sub> are the mouse–human chimeric and murine mAb fragments of the parent intact murine mAb 7E3 [Trikha et al., 2002; Varner et al., 1999]. C7E3 Fab not only binds to  $\alpha_{IIb}\beta_3$  receptor on platelets, which is the major receptor involved in platelet aggregation, but also binds with equivalent affinity to the integrin  $\alpha_v\beta_3$  and, more importantly, it can redistribute between the two receptors in vitro [Tam et al., 1998]. The c7E3 Fab was approved for use as adjunct therapy for the prevention of cardiac ischemic complications in patients undergoing percutaneous coronary intervention [Cohen et al., 2000]. In an in vitro angiogenesis assay, c7E3 Fab inhibited  $\alpha_v\beta_3$ -mediated human umbilical vein endothelial (HUVEC) and melanoma cell adhesion, migration, and invasion, and bFGF stimulated proliferation of HUVECs [Trikha et al., 2002; Varner et al., 1999]. In an animal study, m7E3 F(ab')<sub>2</sub> partially inhibited the growth of human melanoma tumors in nude mice and completely blocked the human melanoma tumor formation and growth in nude rats [Alghisi and Raegg, 2006; Trikha et al., 2002]. Overall, the results suggest that the combined blockade of  $\alpha_{IIb}\beta_3$  and  $\alpha_v\beta_3$  integrins would produce greater anti-angiogenic and anti-tumor benefits than monospecific  $\alpha_v\beta_3$  antagonists alone, given that tumor growth and angiogenesis involve multiple integrin receptors.

**17E6**—The 17E6 antibody, which was raised against purified human  $\alpha_v\beta_3$ , can react with at least  $\alpha_v\beta_3$ ,  $\alpha_v\beta_5$ , and  $\alpha_v\beta_1$  [Mitjans et al., 1995]. 17E6 can strongly perturb cell attachment mediated by  $\alpha_v$  integrins and inhibit cell attachment to  $\alpha_v$  ligands such as vitronectin and fibronectin. Using a panel of six human melanomas and five carcinomas, 17E6 efficiently blocked the in vivo tumor growth of integrin  $\alpha_v\beta_3$ -positive xenografts but did not affect the  $\alpha_v\beta_3$ -negative xenografts [Mitjans et al., 2000; Mitjans et al., 1995]. Given that mAb 17E6 also does not recognize murine  $\alpha_v\beta_3$ , the anti-tumor effect is likely due to the direct anti-tumor activity, and not to the anti-host vasculature activity in the mouse model. In a recent study, it was reported that 17E6 can inhibit the HIV-1 infection of primary macrophages, and the effect of 17E6 on HIV-1 BaL replication in acutely infected macrophages was dose-dependent, suggesting that the  $\alpha_v$  subunit of integrin may be a pharmacological target for HIV infection in macrophages [Bosch et al., 2006].

## DISINTEGRIN, PEPTIDES, AND OTHER ANTAGONISTS

Besides antibodies, there are many other types of antagonists that specifically bind to integrin  $\alpha_v\beta_3$ , such as disintegrins, peptides (mostly RGD-based) and nonpeptidic molecules. Many reports have been published on cancer therapy involving these integrin  $\alpha_v\beta_3$  antagonists, and some have advanced to clinical trials (Table 1).

## Disintegrin

Disintegrin, a family of low molecular weight (47–84 amino acids) RGD containing cysteine-rich peptides derived from viper venoms, can bind specifically to RGD-dependent integrins (such as  $\alpha_v\beta_3$  and  $\alpha_{IIb}\beta_3$ ) and block its function [Huang, 1998]. The first reported disintegrin was the snake venom protein trigramin [Huang et al., 1987]. Since then, many more members of this protein family have been discovered and isolated, such as kistrin [Dennis et al., 1990], bitistatin [Shebuski et al., 1989], barbourin [Scarborough et al., 1991], echistatin [Gan et al., 1988], and contortrostatin [Swenson et al., 2004]. Because of their high integrin binding affinities and their inhibition efficacy, several disintegrins and other integrin inhibiting snake venom components have been evaluated in pre-clinical tests for their capacity to inhibit tumor growth and progression both in vitro and in vivo [Calvete et al., 2003; McLane et al., 2001; Zhou et al., 2004]. However, as proteins they would also elicit an immunological reaction in the patient. In addition, because they are naturally occurring proteins and more prone to enzymatic degradation, their potential clinical applications may be significantly limited. Swenson et al. [2004] reported an effective method for delivery of the snake venom disintegrin contortrostatin (CN) using liposomal system, in an orthotopic xenograft model of human breast cancer in mice. The liposomal formulation of CN (LCN) not only displayed a prolonged circulatory half-life, but also showed no platelet reactivity and no immune system response. Minea et al. [2005] also described a method amenable to large-scale production of a soluble form of recombinant CN (rCN) directly in the cytoplasm of an engineered bacterial system, and the liposomal formulation of the rCN demonstrated potent anti-tumor and anti-angiogenic activities in the MDA-MB-435 human breast cancer xenograft model. Therefore, modification and optimization of disintegrin are typically needed to make them more suitable for clinical translation.

## Peptides

Synthetic peptide-based integrin inhibitors that mimic the structure of the natural ligands of integrins are another of the major aims in the development of integrin-targeting pharmaceuticals. Among the integrin inhibitors, the RGD mimetics are the best known. The RGD triple-peptide, however, is limited in vivo use because of its short circulation half-life. Fortunately, conformational restriction by ring closure of the peptides and further chemical modification, including the use of D-amino acids such as the c(RGDfV) (with f standing for D-phenylalanine) compound, not only could increase their  $\alpha_v\beta_3$  binding affinity but also their bioavailability [Eble and Haier, 2006]. A head-to-tail cyclized RGD-containing pentapeptide, c(RGDf[NMe]V) (also known as Cilengitide or EMD 12, 1974, Merck KGaA, Darmstadt, Germany; Fig. 3), can bind to integrin  $\alpha_v\beta_3$  ( $IC_{50} = 2.3$  nM) and  $\alpha_v\beta_5$  ( $IC_{50} = 37$  nM) with high affinity [Ruoslahti and Pierschbacher, 1986]. Cilengitide has shown positive antiangiogenic effects in vitro [Nisato et al., 2003] and antitumor effects against melanoma, as well as head and neck cancer, breast cancer and brain tumor [Burke et al., 2002; Raguse et al., 2004; Smith, 2003; Taga et al., 2002]. Results of a phase I trial in 51 patients with recurrent malignant glioma demonstrated well tolerated doses of 2,400 mg/m<sup>2</sup>, and stable disease was observed in four patients [Nabors et al., 2007]. Cilengitide is currently in phase II clinical trial for the treatment of highly vascularized head and neck tumors [Raguse et al., 2004].

## Peptidomimetics and Others

There is another class of integrin antagonists named peptidomimetics made either from chemical synthesis or screening from peptidomimetic library. Peptidomimetics are compounds containing nonpeptidic structural elements that are capable of mimicking the biological actions of a natural parent peptide. The important advantages of peptidomimetics are their ability to be amplified and screened from peptidomimetics libraries to identify the

most potent antagonists. Also, because peptidomimetics lack peptide characteristics such as enzymatically scissile peptidic bonds, they are less sensitive to protease-mediated degradation and more suitable for oral availability.

Monsanto (St. Louis, MO) reported an orally bioavailable compound SC-68448 (Fig. 3), which was 100-fold more potent as a functional inhibitor of  $\alpha_v\beta_3$  than  $\alpha_{IIb}\beta_3$  [Carron et al., 2000]. In a preclinical model, SC-68448 inhibited Leydig tumor growth in a dose-dependent manner by up to 80% and completely blocked the development of hypercalcemia [Carron et al., 1998]. SCH221153 (Fig. 3), a compound screened from an RGD-based peptidomimetic library, is a dual inhibitor targeting both the  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  integrins. It showed high affinity to  $\alpha_v\beta_3$  ( $IC_{50} = 3.2$  nM) and  $\alpha_v\beta_5$  ( $IC_{50} = 1.7$  nM), and lower affinity to  $\alpha_{IIb}\beta_3$  ( $IC_{50} = 1294$  nM) and  $\alpha_5\beta_1$  ( $IC_{50} = 421$  nM), and is thus highly selective for both of the two  $\alpha_v$ -dependent integrins [Kumar et al., 2001]. Dual antagonists of both  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  may have enhanced therapeutic advantages, because integrin  $\alpha_v\beta_5$  also plays a critical role in angiogenesis [Friedlander et al., 1995].

## GENE INHIBITORS AND OTHERS

The introduction of small interfering RNA (siRNA) has brought a new approach to the silencing of gene expression, with profound implications for the intervention of human diseases including cancer [Fuchs and Borkhardt, 2007; Lu and Woodle, 2008]. The siRNA oligonucleotides are associated with a multicomponent nuclease RNA-induced silencing complex, which targets and degrades mRNA complementary to the siRNA base sequence. It was reported that siRNA oligonucleotides targeted either the  $\alpha$  or  $\beta$  subunit of the  $\alpha_6\beta_4$  integrin reduced cell surface expression of this integrin and reduced the invasion of MDA-MB-231 breast carcinoma cells, indicating that the siRNA silencing of integrin  $\alpha_6\beta_4$  could be effective in preventing carcinoma cell progression [Lipscomb et al., 2003]. We recently found that silencing  $\alpha_v$  integrin expression by siRNA can inhibit proliferation and induce apoptosis in MDA-MB-435 human breast cancer cells. Importantly, effective reduction of  $\alpha_v$  integrin expression with siRNA can significantly increase the radiosensitivity of tumor cells [Cao et al., 2008].

Recently, several groups have demonstrated that siRNA can also inhibit gene expression in vivo. For example, annealed 21-nucleotide siRNA for luciferase was administered into mice, in combination with a luciferase expression vector. At 72 h later, after giving D-luciferin intraperitoneally, mice receiving luciferase siRNA emitted significantly less light than reporter alone control; that is, siRNA significantly reduced luciferase expression in adult mice [McCaffrey et al., 2002]. The successful applications of siRNA in vivo indicate that RNAi may be a viable approach to treat human diseases including cancer, and siRNA against integrin  $\alpha_v\beta_3$  may have a bright future as it takes effect by eliminating the source directly, rather than merely blocking its biological function.

## COMBINATION THERAPIES

It is conceivable that to be effective clinically, integrin antagonists will have to be used in combination with other antiangiogenic agents or standard anticancer regimens such as chemotherapy or radiotherapy. However, it is unclear which combinations will prove most effective. Previously, combining the anti-angiogenic therapy with other therapeutic modalities such as chemotherapy was considered paradoxical, because anti-angiogenic therapy was assumed to “starve” the tumor cells by destroying the tumor vasculature, thereby depriving the tumor of oxygen and nutrients, whereas chemotherapy needs the blood supply to deliver the drugs inside of the tumor. In recent years, there is emerging evidence to support an alternative hypothesis that certain anti-angiogenic agents can transiently

normalize the abnormal structure and function of tumor blood vessel, leading to more efficient delivery of therapeutics and nutrients [Jain, 2001, 2005] (Fig. 4).

It has been reported in an experimental breast cancer mouse model that the administration of Cilengitide resulted in increased efficacy (53%) of radioimmunotherapy using yttrium-90 labeled antitumor antibody ChL6 as compared with mice treated with  $^{90}\text{Y}$ -ChL6 peptide only [Burke et al., 2002]. Abdollahi et al. [2005] recently showed that the combined treatment with S247, an RGD peptidomimetic antagonist of  $\alpha_v\beta_3$  integrin (Fig. 3), and external beam radiotherapy, enhanced antiangiogenic and anti-tumor effects in glioma (U87MG), epidermoid (A431), and prostate cancer (PC3) xenograft models in nude mice. Other researchers also reported the successful combination of integrin  $\alpha_v\beta_3$  antagonists with chemotherapies for tumor therapy, such as Cilengitide combined with anti-cancer drug gemcitabine for head and neck cancer therapy [Colomer, 2004]. After being treated with Cilengitide on day 1 and 4 in combination with gemcitabine administered days 1 and 8 every 3 weeks for five months, the patient could eat and smell better and remained stable for 12 months on Cilengitide maintenance therapy, with no tendency towards spontaneous bleeding. Abegrin was combined with dacarbazine (DTIC) for a Phase II study involving 112 patients with stage IV metastatic melanoma [Hersey, 2005]. The combined treatment had a 9.4-month median survival period, slightly longer than patients treated with DTIC alone, the current standard of care in advanced melanoma (7.9-month median survival time).

With the wider acceptance of the tumor vasculature normalization concept, more studies on combination therapy are expected to be initiated. The critical challenge will be to define the best combinations in term of drug association, timing, and schedule. In addition, combining the integrin inhibition agents with other antiangiogenic drugs is another highly promising approach. For instance, the combination of EMD270179 (cRGDf-ACHA, ACHA =  $\alpha$ -amino cyclohexyl carboxylic acid), a cyclic RGD based integrin  $\alpha_v$  inhibitor, and SU5416 (a VEGFR-2 kinase inhibitor), showed significantly stronger inhibition of subcutaneous tumor growth and metastasis formation [Strieth et al., 2006]. As a result of the crosstalk and interactions of the different angiogenic molecules such as integrins, VEGF, FGF, EGFR, it will be important to test whether the inhibition of integrin may sensitize the tumor cells to other antiangiogenic drugs.

## INTEGRIN $\alpha_v\beta_3$ -TARGETED DRUG DELIVERY

To achieve an efficient concentration of therapeutics in the tumor, high dose of drugs are typically needed; this can lead to higher system toxicity in patients. For instance, targeted therapy using the specific binding ligand may significantly reduce the drug dose and hence the toxicity risk. In particular, overexpression of integrin  $\alpha_v\beta_3$  on angiogenic tumor vasculature and certain tumor cells makes it an excellent vehicle for delivery of cancer therapeutics such as chemodugs, radioisotopes, and gene inhibitors.

We recently evaluated the antitumor activity of paclitaxel conjugated with a dimeric RGD peptide E[c(RGDyK)]<sub>2</sub> (RGD2) [Cao et al., 2008; Minea et al., 2005]. Paclitaxel (PTX), a prototype of the taxane family of antitumor drugs, is now commonly used in the treatment of advanced metastatic breast cancer. The RGD2-PTX conjugate inhibited cell proliferation with activity comparable to that observed for paclitaxel, both of which are mediated by an arrest of G2/M phase of the cell cycle followed by apoptosis. In addition, when RGD2-PTX was labeled with  $^{125}\text{I}$  through the tyrosine residue on the RGD peptide, integrin-specific accumulation of  $^{125}\text{I}$ -RGD2-PTX in orthotopic MDA-MB-435 tumor was observed. In a followup study, we evaluated the in vivo anti-tumor effect of the RGD2-PTX conjugate [Cao et al., 2008]. The treatment efficacy of RGD2-PTX was confirmed by size measurement, in vivo PET imaging, and ex vivo histopathology. The tumor growth delay is

related to tumor proliferation rather than tumor metabolism, as confirmed by  $^{18}\text{F}$ -FDG/PET and  $^{18}\text{F}$ -FLT/PET.

Burkhart et al. [2004] designed two RGD-based (RGD-4C and Cilengitide) conjugates with the prodrug of doxorubicin, another effective chemotherapeutic for the treatment of a variety of solid tumors including breast tumors. Both conjugates maintained a high affinity for  $\alpha_v\beta_3$  ( $\text{IC}_{50} = 10 \text{ nM}$  and  $5 \text{ nM}$ , respectively). The in vitro cytotoxicity studies revealed good growth inhibition of MDA-MB-435 breast cancer cells, with the  $\text{IC}_{50}$  at  $50 \text{ nM}$  and  $90 \text{ nM}$  for the two conjugates, respectively. No in vivo anti-tumor efficacy of the two conjugates was reported.

Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) is a multi-functional cytokine playing a key role in apoptosis and cell survival, as well as in inflammation and immunity. TNF- $\alpha$  has been fused with peptides containing the NGR or the RGD motif by recombinant DNA technology to enhance the anti-tumor effect and reduce the system damage [Bertilaccio et al., 2008; Corti and Ponzoni, 2004; Sacchi et al., 2004; Van Laarhoven et al., 2006; Zarovni et al., 2004]. We recently developed an RGD4C-TNF fusion protein for tumor-specific delivery of TNF [Wang et al., 2008]. RGD4C-TNF was significantly more potent than TNF in inhibiting orthotopic MDA-MB-435 tumor growth. Intramuscular administration of plasmid DNA encoding RGD-4C-TNF also was found to inhibit the growth of melanomas and lymphomas implanted at sites distant from the plasmid injection site [Zarovni et al., 2004]. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is an apoptosis-inducing member of the TNF gene family, which acts as a homotrimer interacting with five cognate receptors: TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, and osteoprotegerin (OPG). Unlike the other TNF family members TNF- $\alpha$  and CD95L, TRAIL has the unique ability to elicit apoptotic cell death in a variety of cancer cells, with minimal cytotoxicity for normal cells and tissues. Cao et al. [Cao et al., 2008] reported that the fusion of TRAIL to the RGD-based peptide ACDCRGDCFC led to enhanced apoptosis inducing activity (caspase-3 and caspase-8 activation) in  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  integrin-positive cancer cells.

Integrin  $\alpha_v\beta_3$ -targeted delivery of radionuclide has also been reported.  $^{90}\text{Y}$  and  $^{177}\text{Lu}$  complexes ( $^{90}\text{Y}$ -TA138 and  $^{177}\text{Lu}$ -TA138) of a DOTA-conjugated non-peptide integrin  $\alpha_v\beta_3$  antagonist TA138 were prepared.  $^{90}\text{Y}$ -TA138 in the mouse model demonstrated a slowing of tumor growth at a dose of  $15 \text{ mCi/m}^2$ , and a regression of tumors at a dose of  $90 \text{ mCi/m}^2$  [Harris et al., 2003]. Janssen et al. [2002] also reported  $^{111}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ , and  $^{90}\text{Y}$ -labeled cyclic RGD dimer for tumor integrin-targeted imaging and therapy. Injection of  $^{90}\text{Y}$ -DOTA-E-[c(RGDfK)]<sub>2</sub> induced a significant delay in tumor growth. Compared with RGD-based peptides, antibodies seem to be more efficient for tumor radioimmunotherapy (RIT) due to greater tumor uptake and longer tumor residence time. We have evaluated  $^{90}\text{Y}$ -labeled Abegrin in a U87MG glioblastoma xenograft model.  $^{90}\text{Y}$ -Abegrin showed partial tumor regression with a final fractional tumor volume ( $V_{\text{final}}/V_{\text{initial}}$ ) of 0.69, as compared with that of 3.76 for  $^{90}\text{Y}$ -hIgG and 5.43 for normal Abegrin controls, respectively [Veeravagu et al., in press].

Hood et al. [2002] reported the feasibility of selectively deliver inga gene of interest to angiogenic blood vessels in tumor-bearing mice by using a cationic nanoparticle (NP) coupled to an integrin  $\alpha_v\beta_3$ -targeting ligand. The therapeutic efficacy of this approach was tested by generating nanoparticles conjugated to a mutant Raf gene, which blocks endothelial signaling pathway and angiogenesis in response to multiple growth factors. Systemic injection of the nanoparticles into mice induced the apoptosis of the tumor-associated endothelium, ultimately leading to tumor cell apoptosis and sustained regression of established primary and metastatic tumors. Another integrin  $\alpha_v\beta_3$ -targeted gene delivery system was developed for siRNA delivery [Schiffelers et al., 2004]. Self-assembling



nanoparticles with siRNA were constructed with polyethyleneimine (PEI) that is PEGylated with RGD peptide attached at the distal end of the polyethylene glycol (PEG). They were designed to target tumor neovasculature expressing integrins and deliver siRNA that could inhibit vascular endothelial growth factor receptor-2 (VEGFR2) expression and thereby tumor angiogenesis. Intravenous administration of the RGD-PEG-PEI/siRNA complex into tumor-bearing mice exhibited selective tumor uptake, siRNA sequence-specific inhibition of protein expression within the tumor, and inhibition of both tumor angiogenesis and growth rate. These experiments demonstrate the feasibility of tumor-targeted delivery of genes by exploiting the ability of integrins to limit intracellular uptake and reduce the blood stability of gene inhibitors.

## CONCLUSIONS AND PERSPECTIVES

A growing body of preclinical and clinical evidence indicates that integrin  $\alpha_v\beta_3$  is a promising target for antiangiogenic therapy. We summarized here the integrin  $\alpha_v\beta_3$ -targeted therapeutic approaches such as the direct signaling inhibition using antibodies, peptides, peptidomimetics, and other antagonists, as well as indirect tumor-targeted drug delivery systems.

Since integrin  $\alpha_v\beta_3$  is not the only molecule involved in tumor angiogenesis, the combined inhibition of integrin  $\alpha_v\beta_3$  with other angiogenic molecules such as VEGF, FGF, EGF, and other integrins is expected to generate significantly greater anti-angiogenic and anti-tumor benefits over monospecific  $\alpha_v\beta_3$  antagonists. Additionally, the inhibition of integrin  $\alpha_v\beta_3$  combined with other therapeutic modalities, such as chemotherapy and radiotherapy, should be much more effective than using integrin  $\alpha_v\beta_3$  alone. However, the specific synergistic interactions among different modalities should be investigated and clearly understood first to make possible the personalized treatment protocols that will likely become the future of medicine.

Gene therapy is a fundamental way to treat cancer in so much as cancer is a genetic disease. The targeted delivery of siRNA and other gene inhibitors is and will continue to be a vitally important research area for anti-angiogenic cancer therapy in the future. To that end, novel integrin antagonists with high selectivity and affinity produced by chemical synthesis or biological production must be developed and evaluated, moving from preclinical studies to clinical trials. To advance the field, researchers must redouble their efforts to create more tailored agents with improved pharmacokinetics and pharmacodynamics, as well as establishing suitable routes of administration (e.g., oral available) for clinical applications.

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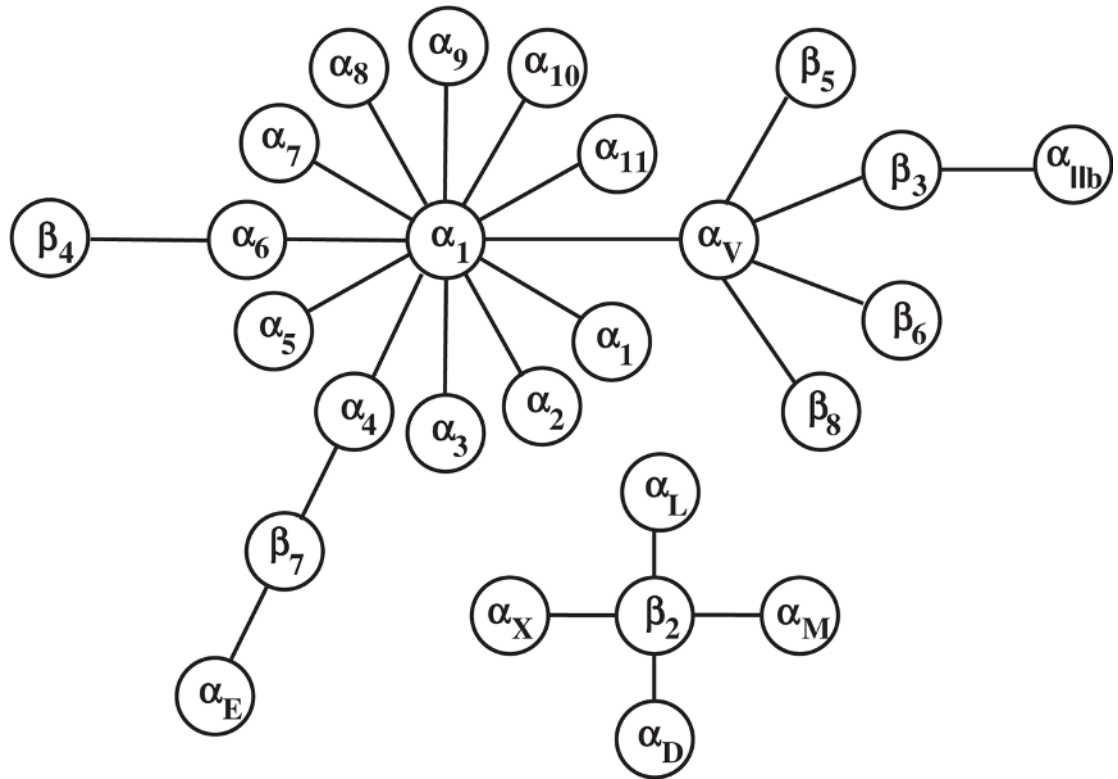
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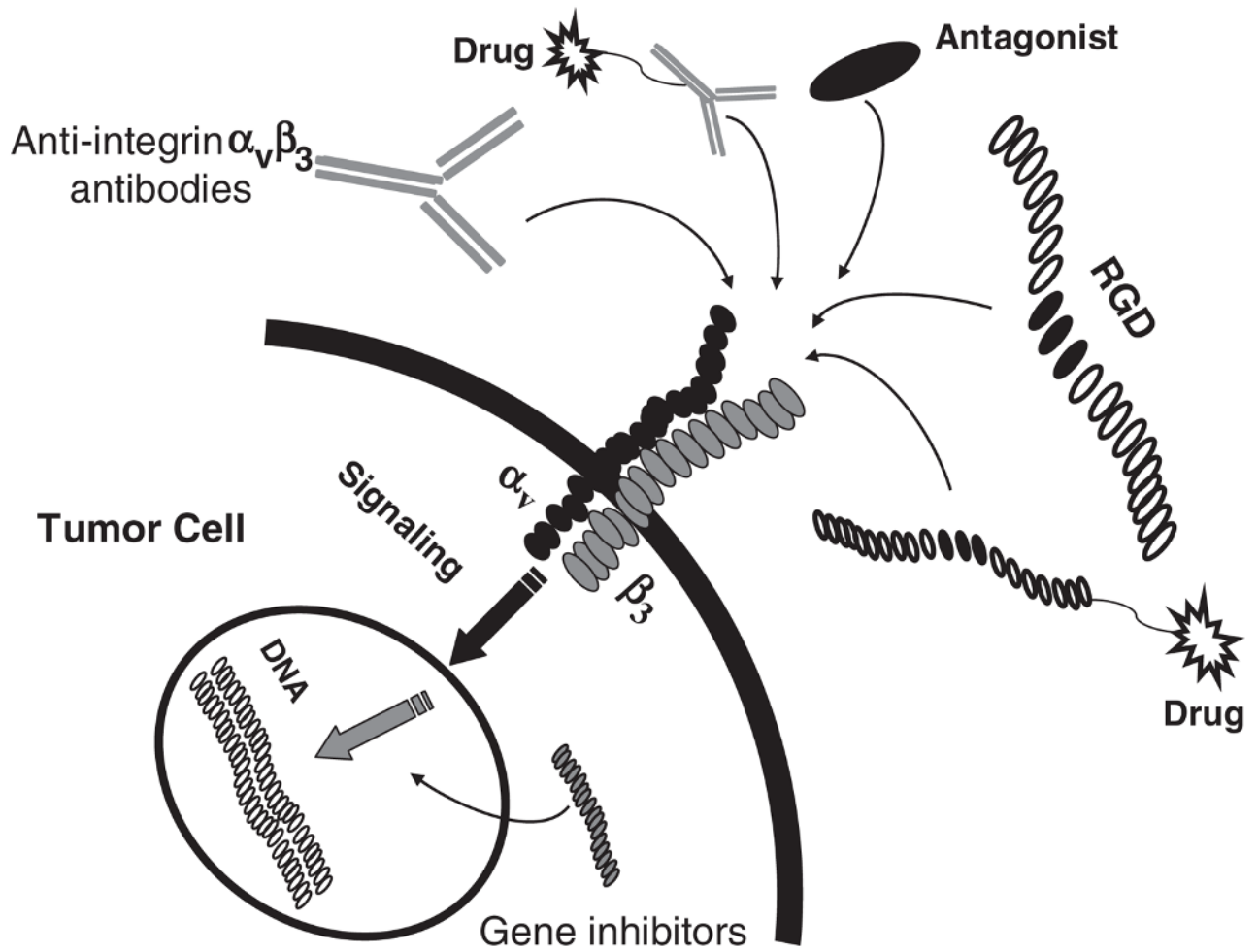
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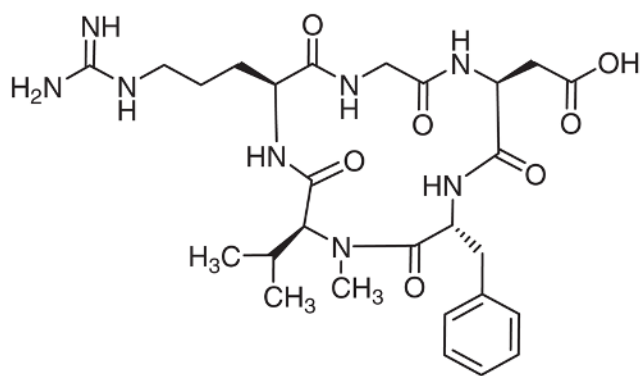


**Fig. 1.**  
The integrin family: 24 heterodimers composed of 18  $\alpha$  and 8  $\beta$  subunits.

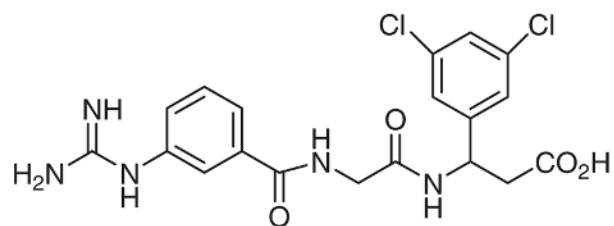


**Fig. 2.** Various strategies to inhibit integrin  $\alpha_v\beta_3$  expression (including direct signaling inhibition and targeted therapeutics).

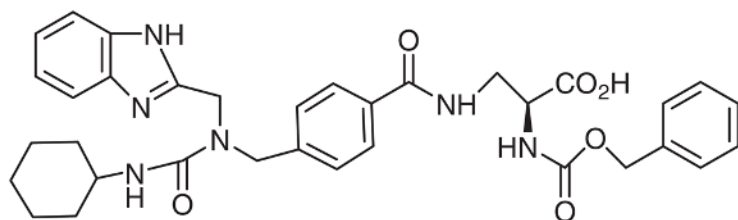




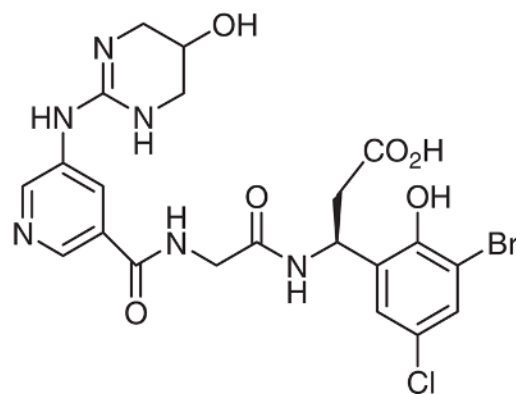
Cilengitide



SC-68448

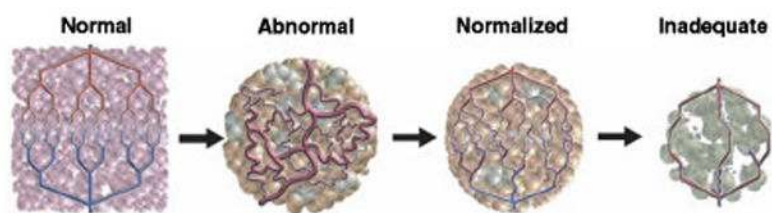


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**Fig. 3.** Schematic structures of a few representative integrin  $\alpha_v\beta_3$  antagonists.



**Fig. 4.** Scheme of changes in tumor vasculature during the course of anti-angiogenic therapy. Adapted with permission from ref. [Jain, 2005]. [Color figure can be viewed in the online version]

TABLE 1

Integrin  $\alpha_v\beta_3$  inhibitors in clinical trials for cancer therapy

Name	Other identity	Targeted integrins	Clinical status	References
Antibodies				
Abegrin	Vitaxin II, MEDI-522	$\alpha_v\beta_3$	Phase II	[Delbaldo et al., 2008; Patel et al., 2001; Posey et al., 2001]
CNT095		$\alpha_v\beta_3, \alpha_v\beta_5$	Phase I	[Mullamitha et al., 2007]
abciximab	C7E3	$\alpha_{IIb}\beta_3, \alpha_v\beta_3, \alpha_v\beta_2$	Phase III	[Cohen et al., 2000]
Peptides				
Cilengitide	EMD 121974	$\alpha_v\beta_3, \alpha_v\beta_5$	Phase II	[Beekman et al., 2006; Colevas et al., 2004; Hariharan et al., 2007; MacDonald et al., 2008; Nabors et al., 2007; Raguse et al., 2004]
ATN-161	Ac-PHSCN-NH <sub>2</sub>	$\alpha_5\beta_1, \alpha_v\beta_3$	Phase II	[Cianfrocca et al., 2006; Donate et al., 2008]