Integrin signaling modes controlling cell migration and metastasis

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Chapter 5

Integrins: Signaling Disease, and Therapy

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Integrins: Signaling, disease, and therapy

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Abstract

Background: Integrins are a family of transmembrane receptors that mediate cell-cell and cell-matrix adhesion. They are involved in stable cell adhesion and migration of cells. In addition, integrin-mediated interactions modulate the response to most, if not all growth factors, cytokines, and other soluble factors.

Purpose: In this review, we briefly explain how integrins can affect the multitude of signal transduction cascades in control of survival, proliferation, and differentiation. Subsequently, we primarily focus on targeting integrins $\alpha 5\beta 1$ and $\alpha v\beta 3$ in disease and we discuss how antagonists of these integrins, including disintegrins, RGD peptides, small molecules, and function blocking antibodies, may be of therapeutical value either alone or, especially in the treatment of cancer, in combination with existing therapeutical strategies.

Keywords: *Adhesion, treatment, cancer*

Introduction

Integrins are cell surface receptors that mediate interactions with the extracellular matrix (ECM) or with counter-receptors on other cells. They cluster and recruit a large multi-protein complex to cell-ECM or cell-cell junctions, which connects them to the cytoskeleton. In addition, signaling proteins and their substrates accumulate at these sites, which regulate the stability of the adhesions and control cytoskeletal dynamics. Besides their critical role in stable cell adhesion and cell migration, integrin-mediated interactions modulate signaling by various other receptors including receptor tyrosine kinases (RTK), G-protein-coupled receptors, cytokine receptors, and others. Consequently, integrins play important roles in survival, proliferation, and differentiation. They are also implicated in several human diseases and integrin antagonists have been tested in preclinical models for various diseases including inflammation, thrombosis, arthritis, and cancer and some have even entered clinical trials. For the treatment of cancer, the expectation is that these antagonists may increase the efficacy of radio- and chemotherapy.

Integrins

Integrins are heterodimeric transmembrane receptors that bind with their globular head domain to components of the ECM. Some integrins can also bind counter receptors present on other cells, bacterial polysaccharides, or viral coat proteins. Intracellularly, integrins are connected via associated proteins to the actin cytoskeleton. 18 α and 8 β subunits are encoded in the human genome from which 24 different functional integrins are currently known to be generated (van der Flier & Sonnenberg 2001, Hynes 2002). Ligand binding can be regulated through integrin clustering and through modulation of the activity of individual integrins which involves the propagation of conformational changes from the cytoplasmic tails across the membrane towards the ligand-binding region (Liddington & Ginsberg, 2002). Integrins can also activate intracellular signal transduction cascades, a process referred to as 'outside-in signaling'. Integrin-mediated cell adhesion can trigger calcium fluxes, activate tyrosine and serine/threonine protein kinases and inositol lipid metabolism, and regulate the activity of the Rho family of small GTPases (Danen & Yamada 2001).

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Genetic studies in flies, worms, and mice have established important roles for integrins and integrin-associated proteins in the development and maintenance of tissues and in the progression of diseases (De Arcangelis & Georges-Labouesse 2000, Bouvard et al. 2001, Bokel & Brown 2002).

Integrin signaling

Integrins generally contain a short cytoplasmic domain which is devoid of enzymatic activity. Therefore outside-in signaling by integrins largely depends on interactions with neighbouring receptors, adaptor and signaling proteins.

Nevertheless integrin signaling is critically important for regulation of signal transduction pathways through distinct mechanisms (Figure 1):

(1) Integrins and growth factor receptors may activate parallel pathways that synergize at the level of activation of downstream signaling proteins. In this way threshold levels in signaling pathways can be lowered considerably (Chen et al. 1996, Renshaw et al. 1997).

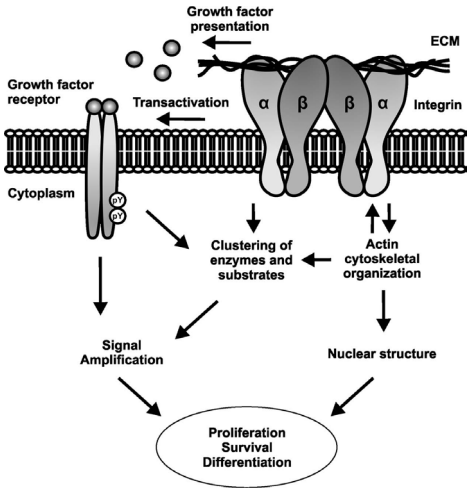

Figure 1. Cross-talk between growth factor receptors and integrins. Integrins affect signal transduction pathways through distinct mechanisms: (1) Together with growth factor receptors, integrins activate parallel pathways that synergize at the level of downstream signaling proteins; (2) Integrins initiate clustering of proteins in cell-matrix adhesions, thereby bringing kinases and substrates in close proximity; (3) Those cell-matrix adhesions also anchor the actin cytoskeleton and thereby generate cytoskeletal tension that affects the nuclear shape and gene expression; (4) Integrin-mediated adhesion can cluster and transactivate receptor tyrosine kinases, and (5) integrins organize the extracellular matrix and thereby regulate upstream signaling of receptors. See text for additional details.

(2) Cell-matrix adhesion initiates clustering of integrins in the plane of the membrane and reorganization of the actin cytoskeleton, which further stimulates the organization of integrins and associated proteins into large multi-protein platforms like focal adhesions, hemidesmosomes or podosomes. In those platforms integrins may increase signals generated by growth factor receptors by bringing kinases and substrates in close proximity (Burridge & Chrzanowska-Wodnicka 1996, Geiger et al. 2001).

(3) Cell-matrix adhesions act as anchoring sites for the actin cytoskeleton and as such they allow the generation of tension and shape changes. Via cytoskeletal connections with the nucleus such changes can affect the nuclear shape and chromatin structure which might explain the profound effect of integrin-mediated cell adhesion on the expression of genes (Maniotis et al. 1997, Lelievre et al. 1998).

(4) Integrin-mediated adhesion can cluster and transactivate several RTK including platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), Ron, Met, and vascular endothelial growth factor receptor (VEGFR) (Yamada & Even-Ram 2002) and members of the Src family kinases (Shattil 2005).

(5) Integrins regulate RTK signaling further upstream through their ability to organize the ECM. Proteoglycans in the ECM can bind and structurally modify various growth factors and integrin-mediated cell adhesion then allows their subsequent presentation to growth factor receptors (Faham et al. 1998). Through these mechanisms integrins play an essential role in regulating signaling pathways in control of survival, proliferation, and differentiation both in health and disease.

Survival

Most adherent cell types depend on integrin-mediated adhesion for survival (Giancotti & Ruoslahti 1999, Cordes 2006, Gilcrease 2007). Loss of adhesion causes cells to undergo apoptosis, a process referred to as anoikis (Frisch & Screaton 2001). Likely, anoikis is important to maintain the integrity of tissues by preventing cells from growing at inappropriate sites after losing adhesion from their original surrounding. Integrin-mediated cell adhesion in 2-dimensional culture systems stimulates phosphatidylinositol-3-kinase (PI3K)-mediated protein kinase B (PKB/AKT) activity and B-cell leukemia-2 (Bcl-2) expression which mediates survival signals (Giancotti & Ruoslahti 1999). In absence of serum factors

integrin-mediated adhesion to fibronectin enhances survival by activating c-Jun N-terminal kinase (JNK) in a focal adhesion kinase (FAK) dependent manner (Almeida et al. 2000). Integrin $\alpha 6\beta 4$ ligation also supports nuclear factor κB (NFκB)-mediated survival signals in 3-dimensional cultures of mammary epithelial cells (Weaver et al. 2002). On the other hand, integrins that are not ligand-bound can trigger apoptosis of fully adherent cells by recruitment and activation of caspase-8 suggesting that a given integrin expression profile renders a cell dependent on a specific ECM environment for its survival (Varner et al. 1995, Stupack et al. 2001).

Proliferation

The ability to grow in the absence of cell adhesion is a key property of oncogenically transformed cells. In normal untransformed cells, integrin-mediated cell adhesion regulates the G1 phase of the cell cycle (Assoian & Schwartz 2001). Integrins cooperate with RTK to stimulate the cyclin E/cyclin dependent kinase 2 (cdk2) activity that drives S-phase entry. Multiple different pathways have been described to connect integrins to cell cycle progression. Regulation of cyclin D1 expression, both at the level of gene transcription and protein accumulation, is a key element of the control of cell cycle progression by RTK and integrins. Control of extracellular signal-regulated kinase (ERK) activation can largely explain the transcriptional regulation of cyclin D1 by integrin-mediated adhesion.

Mitogen-stimulation of RTK and integrin-mediated adhesion can each independently stimulate ERK activation. However, only when adherent cells are stimulated with mitogens ERK activity is strong and sustained due to convergence of RTK and integrin signaling at the level of Raf or MAP/ERK kinase (MEK) (Chen et al. 1996, Renshaw et al. 1997).

There are several ways through which integrins can regulate ERK activity (Howe et al. 2002):

(1) Binding of integrins to the ECM stimulates the formation of an active FAK/Src signaling complex at sites of adhesion. Autophosphorylation of FAK at Tyr^{397} following integrin-mediated adhesion creates a binding site for the Src homology 2 (SH2) domain of Src (Schlaepfer & Hunter 1998). Subsequently, Src can phosphorylate other Tyr residues of FAK thereby creating binding sites for downstream effectors. Direct binding of growth factor receptor binding protein-2 (Grb2) to the active FAK/Src complex or indirect binding through Shc stimulates the Grb2-Sos-Ras-Raf-MEK-ERK pathway. Alternatively, Src can phosphorylate the scaffolding protein p130Cas (Crk associated substrate) that is also associated with FAK via its SH3 domain, thereby creating a binding site for the adaptor protein Crk. Either through association with son of sevenless (Sos) or through association with C3G (a guanine-nucleotide exchange factor for the small GTPase Rap-1), the interaction with Crk can result in ERK activation. Integrin-mediated adhesion also stimulates the association of the adaptor protein Nck with p130Cas, creating yet another potential link from p130Cas to ERK activation. Finally, PI(3)K can associate with phosphorylated Tyr^{397} in FAK and it may become activated upon integrin-mediated cell adhesion. PI(3)K may activate ERK through its role as a protein kinase or through modulation of Sos activity via its production of phosphatidylinositol-3,4,5-trisphosphate (PtdInsP3).

(2) Secondly, certain integrin α-subunits are coupled to the Src family kinase Fyn through association with the oligomeric transmembrane protein Caveolin-1 (Guo & Giancotti 2004). Upon integrin ligand binding, Fyn is activated and it subsequently recruits and phosphorylates Shc, creating a link to the Grb2-Sos-Ras-Raf-MEK-ERK pathway.

(3) Finally, integrin-mediated adhesion activates protein kinase C (PKC) and several PKC isoforms can directly activate Raf. Enhanced levels of phospholipids probably can explain the activation of PKC upon integrin-mediated adhesion. Also integrin-mediated adhesion leads to the activation of the p21-activated protein kinases (PAK) through several mechanisms, and PAK can activate both Raf and MEK.

Besides controlling ERK activity, suppression or relocalization of the cyclin dependent kinase inhibitors p21 and p27 by integrin-mediated adhesion also contributes to G1 cell cycle progression. Additionally, integrin-mediated adhesion increases expression of c-Myc through activation of c-Src (Benaud & Dickson 2001). The organization of the actin cytoskeleton by integrins is essential for adhesion-regulated proliferation and integrin-mediated control of the activity of Rho GTPases (enzymes critically involved in actin cytoskeletal organization) is an important aspect of adhesion-mediated regulation of the levels of cyclin D1 and cdk-inhibitors.

Differentiation

Integrin-mediated cell adhesion also regulates the expression of genes related to differentiation. Adhesion to basement membrane components stimulates the synthesis of milk proteins by increasing

phosphorylation of the prolactin receptor in cultured mammary epithelial cells (Li et al. 1987, Edwards et al. 1998). Integrin-mediated adhesion also primes monocytes for inflammatory responses (Haskill et al. 1988, Shi & Simon 2006). Another example of regulation of differentiation by integrins is the inhibition by integrin-blocking antibodies of the formation of contracting myotubes and expression of meromyosin by embryonic myoblasts (Menko & Boettiger 1987). Deletion of β1 integrins in embryonic stem (ES) cells showed that β1 is important for normal *in vitro* cardiac and myogenic differentiation, whereas neuronal differentiation is accelerated in β1-deficient ES cells (Fassler et al. 1996, Rohwedel et al. 1998). Finally, terminal differentiation of cultured keratinocytes under semi-solid conditions is inhibited by the integrin-ligand fibronectin or by adhesion-blocking antibodies to β1 integrins (Watt 2002). Moreover, a tumor-associated mutation in β1 was recently found that increases ligand binding and prevents terminal differentiation of keratinocytes which might contribute to the formation of epidermal neoplasia (Evans et al. 2003).

Integrins in disease

Aberrant cell adhesion and migration have been implicated in several diseases, including a number of inflammatory disorders such as rheumatoid arthritis, inflammatory bowel disease and asthma, as well as cardiovascular diseases, thrombosis, and cancer. This often correlates with alterations in the expression or functionality of integrins. For instance, deletion of the $\alpha 6\beta 4$ integrin leads to a skin blistering disease termed Epidermolysis bullosa (Borradori & Sonnenberg 1999), deletion of $\alpha 7\beta 1$ causes congenital muscular dystrophy (Vachon et al. 1997), and in patients with Glanzmann's Thrombasthenia platelets fail to aggregate, due to quantitative or qualitative defects of αIIbβ3 (Hodivala-Dilke et al. 1999). On the other hand, in osteoporosis up-regulation of integrin $\alpha v\beta 3$ causes enhanced bone resorption by osteoclasts (Lakkakorpi et al. 1993) and high expression levels of various types of integrins have been correlated with tumor progression in a numbers of cancers (Mizejewski 1999).

The use of integrin antagonists

Integrin-blocking strategies have been developed to treat a large number of diseases (Table I). Integrin antagonists comprise small molecule compounds, peptidomimetics, and monoclonal antibodies (mAb). We will discuss use and action of several drugs with focus on cancer and antagonists of the integrins $\alpha 5\beta 1$ and $\alpha v\beta 3$, being the most extensively studied targets in this disease.

Anti-angiogenesis is an emerging approach for cancer treatment. Angiogenesis, which is the formation of new blood vessels, is a vital process for tumor progression (Folkman 1971). Inhibition of new blood vessel formation has been shown to block the growth and spread of solid tumors in various animal models. There is substantial evidence that several integrins, including $\alpha v\beta 3$, $\alpha v\beta 5$, and $\alpha 5\beta 1$ have an important role in tumor angiogenesis (Brooks et al. 1994a, Friedlander et al. 1995, Kim et al. 2000). The regulation of cell migration and survival of endothelial cells during angiogenesis and metastasis via these integrins makes them suitable targets for anti-angiogenic therapy.

Disintegrins and RGD peptides

The RGD sequence is an important cell attachment recognition site for integrins in many ECM components (Pierschbacher & Ruoslahti 1984, Gardner & Hynes 1985, Plow et al. 1985) and has been used as a pharmaceutical application to treat aberrant cell adhesion related-diseases. Disintegrins are RGD-containing cysteine-rich peptides discovered in snake venoms (Gould et al. 1990). Some disintegrins specifically bind to integrin $\alpha v\beta 3$ and are applied as therapeutic agents for angiogenesis-dependent tumor growth and metastasis (Huang 1998). Others, such as Contortrostatin, which is a disulfide-linked homodimer of 13.5 kDa containing two RGD sites isolated from the venom of *Agkistrodon contortrix*, can bind $\alpha v\beta 3$, $\alpha v\beta 5$, as well as $\alpha 5\beta 1$ and have been shown to inhibit tumor growth and angiogenesis in an orthotopic xenograft model for breast cancer (Swenson et al. 2005).

The disadvantage of natural occurring peptides such as disintegrins is their relatively large size and low metabolic stability, which limits their usefulness for clinical application (McLane et al. 2004, Cai & Chen 2006). RGD peptides have been further optimized by incorporation of D-amino acids and use of cyclic structures. Cyclic RGD-containing pentapeptides are the most commonly used RGD-based $\alpha v\beta 3$ antagonists (Ruoslahti 1996). Cyclo (RGDfV) is a highly effective $\alpha v\beta 3$ antagonist with anti-tumor and anti-angiogenic effects (Brooks et al. 1994b, Friedlander et al. 1995). In a xenograft model for melanoma cyclo (RGDfV)-treatment hindered tumor growth and histological analysis indicated that the effect results from angiogenesis inhibition rather than inhibition of tumor cell $\alpha v\beta 3$ (Dechantsreiter et al. 1999). Systematic modification of this peptide resulted in a more active and selective compound named c(RGDf(NMe)V) also known as Cilengitide (EMD 121974) (Goodman et al. 2002). Cilengitide induces apoptosis in Glioblastoma and

Table I. Integrin antagonists and its targeted diseases.

Integrin target	Drug	Company	Disease	Reference
$\beta 2$	Efalizumab (RabTIVA®)	Genetech	Psoriasis	(Lebwohl et al. 2003)
$\alpha 4$	Natalizumab (Tysabri®)	Biogen Idec & Elan	MS, Crohn's disease	(Miller et al. 2003, Ghosh et al. 2003)
$\alpha 4\beta 1/\alpha 4\beta 7$	TR14035	N/A	Asthma	(Cortijo et al. 2006, Sircar et al. 2002)
$\alpha 4\beta 7$	MLN02	Millennium pharmaceuticals	Ulcerative colitis	(Feagan et al. 2005)
$\alpha 5\beta 1$	Volociximab (M200/Gemzar®)	PDL Biopharma Inc	Renal cell carcinoma, metastastic melanoma, pancreatic cancer	www.clinicaltrials.gov
	Endostatin™	EntreMed Inc.		(O'Reilly et al. 1997)
	ATN-161	N/A		(Stoeltzing et al. 2003, Livant et al. 2000)
$\alpha v\beta 3$	Chimeric 7E3 Fab (Abciximab/ReoPro®)	Centocor		(Trikha et al. 2002)
	Contortrostatin	N/A		(Clark et al. 1994, Trikha et al. 1994)
	C(RGDf(NMe)V) (Cilengitide, EMD 121974)	Merck KGaA	Angiogenesis, cancer	www.cancer.gov/clinicaltrials (Albert et al. 2006, Taga et al. 2002)
	C (RGDfV) cyclo (Arg-Gly-Asp-D-Phe-Val)	N/A		(Allman et al. 2000, Friedlander et al. 1995, Brooks et al. 1994b, Dechantsreiter et al. 1999)
	S247	Pharmacia Corp		(Reinmuth et al. 2003, Abdollahi et al. 2005)
	Resveratrol	N/A		(Aggarwal et al. 2004)
	Vitaxin	MedImmune Inc.		(Mikecz 2000)
	SB273005	SmithKline Beecham Pharmaceuticals	Rheumatoid arthritis	(Badger et al. 2001)
	SC55631	N/A	Osteoporosis	(Engleman et al. 1997)
$\alpha IIb\beta 3$	Chimeric 7E3 Fab (Abciximab/ReoPro®)	Centocor	Unstable angina, restenosis, stroke,	(Bennett 2001)
	Lotrafiban	SmithKline Beecham Pharmaceuticals	acute coronary artery disease	(Liu et al. 2000)

medullablastoma cells (Taga et al. 2002). It is applied in phase I and II to treat non-small lung cancer, prostate cancer, glioblastoma, pancreatic cancer, melanoma, and lymphoma (www.cancer.gov/clinicaltrials).

Small molecule integrin antagonists

There is evidence that small molecule antagonists could be used to treat human diseases, which depend on angiogenesis, including rheumatoid arthritis, osteoporosis, and cancer (Hartman & Duggan 2000, Kerr et al. 2000, Giavazzi & Nicoletti 2002, Shimaoka & Springer 2003). For this purpose, new classes of small molecule $\alpha v\beta 3$ antagonists have been developed, including isoxazolines (Pitts et al. 2000), disubstituted indazoles (Batt et al. 2000), and non-peptide chemical RGD peptidomimetics. An example of this latter class of antagonists, S247, which blocks $\alpha v\beta 3/\alpha v\beta 5$, was shown to decrease tumor growth and angiogenesis of colon cancer liver metastases leading to prolonged survival in an orthotopic murine model (Reinmuth et al. 2003). Another non-peptide chemical RGD mimetic that targets $\alpha v\beta 3$, SC56631 inhibits bone resorption in vitro and suppresses osteoporosis in oestrogen-deprived animals (Engleman et al. 1997). The naturally occurring polyphenol, Stilbene Resveratrol also binds to integrin $\alpha v\beta 3$ at or near the RGD-binding site and induces apoptosis in cancer cells through activation of ERK with consequent phosphorylation of p53 (Lin et al. 2006). Compared to $\alpha v\beta 3$, few small molecule antagonists are known to inhibit $\alpha 5\beta 1$. Nevertheless, treatment with ATN-161, a non-RGD peptide specific for $\alpha 5\beta 1$ that is derived from the synergy site in fibronectin that is part of the $\alpha 5\beta 1$-binding motif but not of that of $\alpha v\beta 3$, enhanced the efficacy of chemotherapy in a mouse model for colon cancer metastasis (Stoeltzing et al. 2003).

Monoclonal antibodies

LM609 is an anti-human integrin $\alpha v\beta 3$ mAb that blocks cell adhesion to RGD-containing ligands (Cheresh 1987). It prevents bFGF (basic fibroblast growth factor)- and TNFα (tumor necrosis factor-α)-dependent angiogenesis but has no effect on pre-existing vessels (Brooks et al. 1994a). LM609 was effective in preclinical models for glioblastoma, melanoma, breast, and prostate cancer. Intravenous administration of LM609 inhibited outgrowth of $\alpha v\beta 3$-negative human breast cancer cells in a combined (SCID) mouse/human chimeric model (Brooks et al. 1995). Fewer human blood vessels and less invasive tumors were observed in these LM609-treated animals with no apparent effect on normal human tissue, indicating that LM609 acts as an anti-angiogenic compound. Similar effects have been obtained with antibodies directed against $\alpha 5\beta 1$ (Kim et al. 2000).

The serum half-life and integrin-binding affinity of LM609 have been improved by generating a humanized version, Vitaxin (Carter 2001, Wu & Senter 2005). Optimization of the complementarity-determining regions further improved Vitaxin's integrin binding affinity allowing it to inhibit tumor growth in Kaposi's sarcoma and partially inhibit the binding of the human immunodeficiency virus-1 (HIV-1) Tat protein to $\alpha v\beta 3$ (Rader et al. 2002). Vitaxin II, although binding $\alpha v\beta 3$ with even higher affinity, showed no positive response in cancer treatment but may be effective as adjuvant in combination with chemo- or radiation therapy (Posey et al. 2001). In 2003, MedImmune licensed Vitaxin II (MEDI-522) for clinical development in phase II trials in prostate cancer, melanoma, psoriasis, and rheumatoid arthritis. However in 2004, the clinical trial of Vitaxin in the treatment of rheumatoid arthritis and psoriasis was ended because preliminary results failed to demonstrate clinical benefits. Nevertheless, the trials for melanoma and prostate cancer are still in progress (MedImmune ends some Vitaxin testing, advanced tests for arthritis treatment halted; cancer research continues. Article by Michael S. Rosenwald, *Washington Post* staff writer; August 31, 2004; Page E05).

Strongly improved versions of antibodies against $\alpha 5\beta 1$ have also been generated. For instance,Volociximab, a chimeric humanized mAb, is a high affinity function inhibitor of the $\alpha 5\beta 1$ integrin, which, like $\alpha v\beta 3$ has been found to be upregulated in activated endothelial cells. It has been applied in clinical phase II trials for solid tumors in renal cell carcinoma, metastatic melanoma and pancreatic cancer. These are ongoing studies with no data reported yet (www.clinicaltrials.gov).

Potential of integrin antagonists to improve efficacy of existing anticancer therapy

Similar to other anti-angiogenic agents, integrin antagonists can be applied in combination with cytotoxic anticancer therapy, such as chemo- or radiotherapy. Such combinational approaches can maximize efficacy in cancer by destroying cancer cells as well as endothelial cells, the latter depriving the tumor of nutrients and oxygen (Teicher 1996). On the other hand, the anti-angiogenic agent may also 'normalize' the abnormal structure and function of tumor vessels, thereby improving drug (but also oxygen) delivery (Jain 2005). Examples of such combination therapies are discussed in the following (Figure 2).

The ATN-161 $\alpha 5\beta 1$ antagonist enhanced the efficacy of chemotherapy in a mouse model for colon cancer metastasis (Stoeltzing et al. 2003). Co-application with Cilengitide increased the anti-tumor effectiveness of a tumor-specific antibody against interleukin 2 (IL-2) fusion proteins in a murine tumor model (melanoma, colon carcinoma, and neuroblastoma) (Lode et al. 1999). In pancreatic cancer, Cilengitide combined with gemcitabine (a radiation-sensitizing agent and a wide spectrum anti-cancer drug) inhibited highly vascularized tumor growth (Colomer 2004, Raguse et al. 2004). In ongoing trials for breast cancer, colon cancer, prostate cancer, melanoma, lung cancer, glioblastoma, and ovarian cancer, Vitaxin II is applied in combination with chemo-, hormonal-, biological-, immuno-, or radiotherapy. Patients with Stage IV

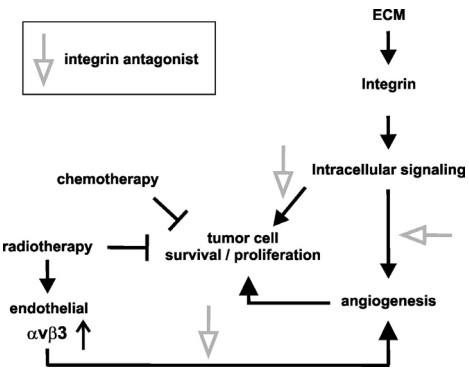

Figure 2. Integrins as targets in anti-cancer therapy. Open arrows indicate processes that are blocked by integrin antagonists. These include survival and proliferation of tumor cells as well as survival, proliferation, and/or migration of endothelial cells. The latter may be of particular importance during radiotherapy where increased expression of $\alpha v\beta 3$ on endothelial cells can mediate therapy escape through enhanced angiogenesis.

melanoma have 9.4-month median survival when treated with Vitaxin II combined with dacarbazine (DTIC) whereas patients treated with DTIC alone, have a median survival of 7.9 months (Cai & Chen 2006). It has been reported that radiotherapy in fact promotes integrin-mediated survival signaling through PKB/Akt by upregulating $\alpha v\beta 3$ expression on endothelial cells. This escape mechanism can be circumvented by administering angiogenesis inhibitors, such as the small molecule $\alpha v\beta 3$ integrin antagonist S247, which prevents radiation-induced PKB/Akt phosphorylation leading to enhanced anti-angiogenic and anti-tumor effects (Abdollahi et al. 2005).

Most integrin antagonists interfere with binding of natural ECM components to their receptors and thereby prevent integrin signaling to survival and proliferation. As such, integrin antagonists may effectively suppress tumorigenesis by targeting these signaling pathways in both tumor and endothelial cells. Ultimately, a trimodal strategy in which radio-, chemo-, and anti-angiogenic therapies are combined may be highly effective in the treatment of cancer. The use of integrin antagonists as anti-angiogenic agents that may also target tumor cells, may fit well in such strategies.

References

Abdollahi A, Griggs DW, Zieher H, Roth A, Lipson KE, Saffrich R, Grone HJ, Hallahan DE, Reisfeld RA, Debus J, Niethammer AG, Huber PE. 2005. Inhibition of alpha(v)beta3 integrin survival signaling enhances antiangiogenic and antitumor effects of radiotherapy. Clinical Cancer Research 11:6270 – 6279.

Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. 2004. Role of resveratrol in prevention and therapy of cancer: Preclinical and clinical studies. Anticancer Research 24:2783 – 2840.

Albert JM, Cao C, Geng L, Leavitt L, Hallahan DE, Lu B. 2006. Integrin alpha v beta 3 antagonist Cilengitide enhances efficacy of radiotherapy in endothelial cell and non-small-cell lung cancer models. International Journal of Radiation Oncology, Biology, Physics 65:1536 – 1543.

Allman R, Cowburn P, Mason M. 2000. *In vitro* and *in vivo* effects of a cyclic peptide with affinity for the alpha(nu)beta3 integrin in human melanoma cells. European Journal of Cancer 36:410 – 422.

Almeida EA, Ilic D, Han Q, Hauck CR, Jin F, Kawakatsu H, Schlaepfer DD, Damsky CH. 2000. Matrix survival signaling: from fibronectin via focal adhesion kinase to c-Jun NH(2)-terminal kinase. Journal of Cell Biology 149:741 – 754.

Assoian RK, Schwartz MA. 2001. Coordinate signaling by integrins and receptor tyrosine kinases in the regulation of G1 phase cell-cycle progression. Current Opinion in Genetics & Development 11:48 – 53.

Badger AM, Blake S, Kapadia R, Sarkar S, Levin J, Swift BA, Hoffman SJ, Stroup GB, Miller WH, Gowen M, Lark MW. 2001. Disease-modifying activity of SB 273005, an orally active, nonpeptide alphavbeta3 (vitronectin receptor) antagonist, in rat adjuvant-induced arthritis. Arthritis & Rheumatism 44:128 – 137.

Batt DG, Petraitis JJ, Houghton GC, Modi DP, Cain GA, Corjay MH, Mousa SA, Bouchard PJ, Forsythe MS, Harlow PP, Barbera FA, Spitz SM, Wexler RR, Jadhav PK. 2000. Disubstituted indazoles as potent antagonists of the integrin alpha(v)beta(3). Journal of Medicinal Chemistry 43:41 – 58.

Benaud CM, Dickson RB. 2001. Regulation of the expression of c-Myc by beta1 integrins in epithelial cells. Oncogene 20:759 – 768.

Bennett JS. 2001. Novel platelet inhibitors. Annual Review of Medicine 52:161 – 184.

Bokel C, Brown NH. 2002. Integrins in development: Moving on, responding to, and sticking to the extracellular matrix. Developmental Cell 3:311 – 321.

Borradori L, Sonnenberg A. 1999. Structure and function of hemidesmosomes: more than simple adhesion complexes. Journal of Investigative Dermatology 112:411 – 418.

Bouvard D, Brakebusch C, Gustafsson E, Aszodi A, Bengtsson T, Berna A, Fassler R. 2001. Functional consequences of integrin gene mutations in mice. Circulation Research 89:211 – 223.

Brooks PC, Clark RA, Cheresh DA. 1994a. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science 264:569 – 571.

Brooks PC, Montgomery AM, Rosenfeld M, Reisfeld RA, Hu T, Klier G, Cheresh DA. 1994b. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell 79:1157 – 1164.

Brooks PC, Stromblad S, Klemke R, Visscher D, Sarkar FH, Cheresh DA. 1995. Antiintegrin alpha v beta 3 blocks human breast cancer growth and angiogenesis in human skin. Journal of Clinical Investigation 96:1815 – 1822.

Burridge K, Chrzanowska-Wodnicka M. 1996. Focal adhesions, contractility, and signaling. Annual Review of Cell and Developmental Biology 12:463 – 518.

Cai W, Chen X. 2006. Anti-angiogenic cancer therapy based on integrin alphavbeta3 antagonism. Anti-Cancer Agents in Medicinal Chemistry 6:407 – 428.

Carter P. 2001. Improving the efficacy of antibody-based cancer therapies. Nature Reviews Cancer 1:118 – 129.

Chen Q, Lin TH, Der CJ, Juliano RL. 1996. Integrin-mediated activation of MEK and mitogen-activated protein kinase is independent of Ras [corrected]. Journal of Biological Chemistry 271:18122 – 18127.

Cheresh DA. 1987. Human endothelial cells synthesize and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor. Proceedings of the National Academy of Sciences USA 84:6471 – 6475.

Clark EA, Trikha M, Markland FS, Brugge JS. 1994. Structurally distinct disintegrins contortrostatin and multisquamatin differentially regulate platelet tyrosine phosphorylation. Journal of Biological Chemistry 269:21940 – 21943.

Colomer R. 2004. Gemcitabine and paclitaxel in metastatic breast cancer: A review. Oncology (Williston. Park) 18:8 – 12.

Cordes N. 2006. Integrin-mediated cell-matrix interactions for prosurvival and antiapoptotic signaling after genotoxic injury. Cancer Letters 242:11 – 19.

Cortijo J, Sanz MJ, Iranzo A, Montesinos JL, Nabah YN, Alfon J, Gomez LA, Merlos M, Morcillo EJ. 2006. A small molecule, orally active, alpha4beta1/alpha4beta7 dual antagonist reduces leukocyte infiltration and airway hyper-responsiveness in an experimental model of allergic asthma in Brown Norway rats. British Journal of Pharmacology 147:661 – 670.

Danen EH, Yamada KM. 2001. Fibronectin, integrins, and growth control. Journal of Cellular Physiology 189:1 – 13.

De Arcangelis A, Georges-Labouesse E. 2000. Integrin and ECM functions: Roles in vertebrate development. Trends in Genetics 16:389 – 395.

Dechantsreiter MA, Planker E, Matha B, Lohof E, Holzemann G, Jonczyk A, Goodman SL, Kessler H. 1999. N-Methylated cyclic RGD peptides as highly active and selective alpha(V) beta(3) integrin antagonists. Journal of Medicinal Chemistry 42:3033 – 3040.

Edwards GM, Wilford FH, Liu X, Hennighausen L, Djiane J, Streuli CH. 1998. Regulation of mammary differentiation by extracellular matrix involves protein-tyrosine phosphatases. Journal of Biological Chemistry 273:9495 – 9500.

Engleman VW, Nickols GA, Ross FP, Horton MA, Griggs DW, Settle SL, Ruminski PG, Teitelbaum SL. 1997. A peptidomimetic antagonist of the alpha(v)beta3 integrin inhibits bone resorption *in vitro* and prevents osteoporosis *in vivo*. Journal of Clinical Investigation 99:2284 – 2292.

Evans RD, Perkins VC, Henry A, Stephens PE, Robinson MK, Watt FM. 2003. A tumor-associated beta 1 integrin mutation that abrogates epithelial differentiation control. Journal of Cell Biology 160:589 – 596.

Faham S, Linhardt RJ, Rees DC. 1998. Diversity does make a difference: fibroblast growth factor-heparin interactions. Current Opinion in Structural Biology 8:578 – 586.

Fassler R, Rohwedel J, Maltsev V, Bloch W, Lentini S, Guan K, Gullberg D, Hescheler J, Addicks K, Wobus AM. 1996. Differentiation and integrity of cardiac muscle cells are impaired in the absence of beta 1 integrin. Journal of Cell Science 109 (Pt 13):2989 – 2999.

Feagan BG, Greenberg GR, Wild G, Fedorak RN, Pare P, McDonald JW, Dube R, Cohen A, Steinhart AH, Landau S, Aguzzi RA, Fox IH, Vandervoort MK. 2005. Treatment of ulcerative colitis with a humanized antibody to the alpha4beta7 integrin. New England Journal of Medicine 352:2499 – 2507.

Folkman J. 1971. Tumor angiogenesis: therapeutic implications. New England Journal of Medicine 285:1182 – 1186.

Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA. 1995. Definition of two angiogenic pathways by distinct alpha v integrins. Science 270:1500 – 1502.

Frisch SM, Screaton RA. 2001. Anoikis mechanisms. Current Opinion in Cell Biology 13:555 – 562.

Gardner JM, Hynes RO. 1985. Interaction of fibronectin with its receptor on platelets. Cell 42:439 – 448.

Geiger B, Bershadsky A, Pankov R, Yamada KM. 2001. Transmembrane crosstalk between the extracellular matrix – cytoskeleton crosstalk. Nature Reviews Molecular Cell Biology 2:793 – 805.

Ghosh S, Goldin E, Gordon FH, Malchow HA, Rask-Madsen J, Rutgeerts P, Vyhnalek P, Zadorova Z, Palmer T, Donoghue S. 2003. Natalizumab for active Crohn's disease. New England Journal of Medicine 348:24 – 32.

Giancotti FG, Ruoslahti E. 1999. Integrin signaling. Science 285:1028 – 1032.

Giavazzi R, Nicoletti MI. 2002. Small molecules in anti-angiogenic therapy. Current Opinion in Investigational Drugs 3:482 – 491.

Gilcrease MZ. 2007. Integrin signaling in epithelial cells. Cancer Letters 247:1 – 25.

Goodman SL, Holzemann G, Sulyok GA, Kessler H. 2002. Nanomolar small molecule inhibitors for alphav(beta)6, alphav(beta)5, and alphav(beta)3 integrins. Journal of Medicinal Chemistry 45:1045 – 1051.

Gould RJ, Polokoff MA, Friedman PA, Huang TF, Holt JC, Cook JJ, Niewiarowski S. 1990. Disintegrins: a family of integrin inhibitory proteins from viper venoms. Proceedings of the Society for Experimental Biology & Medicine 195:168 – 171.

Guo W, Giancotti FG. 2004. Integrin signalling during tumour progression. Nature Reviews Molecular Cell Biology 5:816 – 826.

Hartman GD, Duggan ME. 2000. alpha(v)beta(3) Integrin antagonists as inhibitors of bone resorption. Expert Opinion on Investigational Drugs 9:1281 – 1291.

Haskill S, Johnson C, Eierman D, Becker S, Warren K. 1988. Adherence induces selective mRNA expression of monocyte mediators and proto-oncogenes. Journal of Immunology 140:1690 – 1694.

Hodivala-Dilke KM, McHugh KP, Tsakiris DA, Rayburn H, Crowley D, Ullman-Cullere M, Ross FP, Coller BS, Teitelbaum S, Hynes RO. 1999. Beta3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. Journal of Clinical Investigation 103:229 – 238.

Howe AK, Aplin AE, Juliano RL. 2002. Anchorage-dependent ERK signaling – mechanisms and consequences. Current Opinion in Genetics & Development 12:30 – 35.

Huang TF. 1998. What have snakes taught us about integrins? Cellular & Molecular Life Sciences 54:527 – 540.

Hynes RO. 2002. Integrins: bidirectional, allosteric signaling machines. Cell 110:673 – 687.

Jain RK. 2005. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 307:58 – 62.

Kerr JS, Slee AM, Mousa SA. 2000. Small molecule alpha(v) integrin antagonists: novel anticancer agents. Expert Opinion on Investigational Drugs 9:1271 – 1279.

Kim S, Bell K, Mousa SA, Varner JA. 2000. Regulation of angiogenesis *in vivo* by ligation of integrin alpha5beta1 with the central cell-binding domain of fibronectin. American Journal of Pathology 156:1345 – 1362.

Lakkakorpi PT, Helfrich MH, Horton MA, Vaananen HK. 1993. Spatial organization of microfilaments and vitronectin receptor, alpha v beta 3, in osteoclasts. A study using confocal laser scanning microscopy. Journal of Cell Science 104 (Pt 3):663 – 670.

Lebwohl M, Tyring SK, Hamilton TK, Toth D, Glazer S, Tawfik NH, Walicke P, Dummer W, Wang X, Garovoy MR, Pariser D. 2003. A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. New England Journal of Medicine 349:2004 – 2013.

Lelievre SA, Weaver VM, Nickerson JA, Larabell CA, Bhaumik A, Petersen OW, Bissell MJ. 1998. Tissue phenotype depends on reciprocal interactions between the extracellular matrix and the structural organization of the nucleus. Proceedings of the National Academy of Sciences USA 95:14711 – 14716.

Li ML, Aggeler J, Farson DA, Hatier C, Hassell J, Bissell MJ. 1987. Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. Proceedings of the National Academy of Sciences USA 84:136 – 140.

Liddington RC, Ginsberg MH. 2002. Integrin activation takes shape. Journal of Cell Biology 158:833 – 839.

Lin HY, Lansing L, Merillon JM, Davis FB, Tang HY, Shih A, Vitrac X, Krisa S, Keating T, Cao HJ, Bergh J, Quackenbush S, Davis PJ. 2006. Integrin alphaVbeta3 contains a receptor site for resveratrol. FASEB Journal 20:1742 – 1744.

Liu F, Craft RM, Morris SA, Carroll RC. 2000. Lotrafiban: an oral platelet glycoprotein IIb/IIIa blocker. Expert Opinion on Investigational Drugs 9:2673 – 2687.

Livant DL, Brabec RK, Pienta KJ, Allen DL, Kurachi K, Markwart S, Upadhyaya A. 2000. Anti-invasive, antitumorigenic, and antimetastatic activities of the PHSCN sequence in prostate carcinoma. Cancer Research 60:309 – 320.

Lode HN, Moehler T, Xiang R, Jonczyk A, Gillies SD, Cheresh DA, Reisfeld RA. 1999. Synergy between an antiangiogenic integrin alphav antagonist and an antibody-cytokine fusion protein eradicates spontaneous tumor metastases. Proceedings of the National Academy of Sciences USA 96:1591 – 1596.

Maniotis AJ, Chen CS, Ingber DE. 1997. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. Proceedings of the National Academy of Sciences USA 94:849 – 854.

McLane MA, Sanchez EE, Wong A, Paquette-Straub C, Perez JC. 2004. Disintegrins. Current Drug Targets – Cardiovascular & Haematological Disorders 4:327–355.

Menko AS, Boettiger D. 1987. Occupation of the extracellular matrix receptor, integrin, is a control point for myogenic differentiation. Cell 51:51–57.

Mikecz K. 2000. Vitaxin applied molecular evolution. Current Opinion in Investigational Drugs 1:199–203.

Miller DH, Khan OA, Sheremata WA, Blumhardt LD, Rice GP, Libonati MA, Willmer-Hulme AJ, Dalton CM, Miszkiel KA, O'Connor PW. 2003. A controlled trial of natalizumab for relapsing multiple sclerosis. New England Journal of Medicine 348:15–23.

Mizejewski GJ. 1999. Role of integrins in cancer: survey of expression patterns. Proceedings of the Society for Experimental Biology & Medicine 222:124–138.

O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. 1997. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 88:277–285.

Pierschbacher MD, Ruoslahti E. 1984. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature 309:30–33.

Pitts WJ, Wityak J, Smallheer JM, Tobin AE, Jetter JW, Buynitsky JS, Harlow PP, Solomon KA, Corjay MH, Mousa SA, Wexler RR, Jadhav PK. 2000. Isoxazolines as potent antagonists of the integrin alpha(v)beta(3). Journal of Medicinal Chemistry 43:27–40.

Plow EF, Pierschbacher MD, Ruoslahti E, Marguerie GA, Ginsberg MH. 1985. The effect of Arg-Gly-Asp-containing peptides on fibrinogen and von Willebrand factor binding to platelets. Proceedings of the National Academy of Sciences USA 82:8057–8061.

Posey JA, Khazaeli MB, DelGrosso A, Saleh MN, Lin CY, Huse W, LoBuglio AF. 2001. A pilot trial of Vitaxin, a humanized anti-vitronectin receptor (anti alpha v beta 3) antibody in patients with metastatic cancer. Cancer Biotherapy & Radiopharmaceuticals 16:125–132.

Rader C, Popkov M, Neves JA, Barbas CF III. 2002. Integrin alpha(v)beta3 targeted therapy for Kaposi's sarcoma with an in vitro evolved antibody. FASEB Journal 16:2000–2002.

Raguse JD, Gath HJ, Bier J, Riess H, Oettle H. 2004. Cilengitide (EMD 121974) arrests the growth of a heavily pretreated highly vascularised head and neck tumour. Oral Oncology 40:228–230.

Reinmuth N, Liu W, Ahmad SA, Fan F, Stoeltzing O, Parikh AA, Bucana CD, Gallick GE, Nickols MA, Westlin WF, Ellis LM. 2003. Alphavbeta3 integrin antagonist S247 decreases colon cancer metastasis and angiogenesis and improves survival in mice. Cancer Research 63:2079–2087.

Renshaw MW, Ren XD, Schwartz MA. 1997. Growth factor activation of MAP kinase requires cell adhesion. EMBO Journal 16:5592–5599.

Rohwedel J, Guan K, Zuschratter W, Jin S, Hnert-Hilger G, Furst D, Fassler R, Wobus AM. 1998. Loss of beta1 integrin function results in a retardation of myogenic, but an acceleration of neuronal, differentiation of embryonic stem cells *in vitro*. Developmental Biology 201:167–184.

Ruoslahti E. 1996. RGD and other recognition sequences for integrins. Annual Review of Cell & Developmental Biology 12:697–715.

Schlaepfer DD, Hunter T. 1998. Integrin signalling and tyrosine phosphorylation: Just the FAKs? Trends in Cell Biology 8:151–157.

Shattil SJ. 2005. Integrins and Src: Dynamic duo of adhesion signaling. Trends in Cell Biology 15:399–403.

Shi C, Simon DI. 2006. Integrin signals, transcription factors, and monocyte differentiation. Trends in Cardiovascular Medicine 16:146–152.

Shimaoka M, Springer TA. 2003. Therapeutic antagonists and conformational regulation of integrin function. Nature Reviews Drug Discovery 2:703–716.

Sircar I, Gudmundsson KS, Martin R, Liang J, Nomura S, Jayakumar H, Teegarden BR, Nowlin DM, Cardarelli PM, Mah JR, Connell S, Griffith RC, Lazarides E. 2002. Synthesis and SAR of N-benzoyl-L-biphenylalanine derivatives: Discovery of TR-14035, a dual alpha(4)beta(7)/alpha(4)beta(1) integrin antagonist. Bioorganic & Medicinal Chemistry 10:2051–2066.

Stoeltzing O, Liu W, Reinmuth N, Fan F, Parry GC, Parikh AA, McCarty MF, Bucana CD, Mazar AP, Ellis LM. 2003. Inhibition of integrin alpha5beta1 function with a small peptide (ATN-161) plus continuous 5-FU infusion reduces colorectal liver metastases and improves survival in mice. International Journal of Cancer 104:496–503.

Stupack DG, Puente XS, Boutsaboualoy S, Storgard CM, Cheresh DA. 2001. Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. Journal of Cell Biology 155:459–470.

Swenson S, Costa F, Ernst W, Fujii G, Markland FS. 2005. Contortrostatin, a snake venom disintegrin with anti-angiogenic and anti-tumor activity. Pathophysiology of Haemostasis and Thrombosis 34:169–176.

Taga T, Suzuki A, Gonzalez-Gomez I, Gilles FH, Stins M, Shimada H, Barsky L, Weinberg KI, Laug WE. 2002. alpha v-Integrin antagonist EMD 121974 induces apoptosis in brain tumor cells growing on vitronectin and tenascin. International Journal of Cancer 98:690–697.

Teicher BA. 1996. A systems approach to cancer therapy. (Antioncogenics + standard cytotoxics −> mechanism(s) of interaction). Cancer and Metastasis Reviews 15:247–272.

Trikha M, Rote WE, Manley PJ, Lucchesi BR, Markland FS. 1994. Purification and characterization of platelet aggregation inhibitors from snake venoms. Thrombosis Research 73:39–52.

Trikha M, Zhou Z, Timar J, Raso E, Kennel M, Emmell E, Nakada MT. 2002. Multiple roles for platelet GPIIb/IIIa and alphavbeta3 integrins in tumor growth, angiogenesis, and metastasis. Cancer Research 62:2824–2833.

Vachon PH, Xu H, Liu L, Loechel F, Hayashi Y, Arahata K, Reed JC, Wewer UM, Engvall E. 1997. Integrins (alpha7beta1) in muscle function and survival. Disrupted expression in merosin-deficient congenital muscular dystrophy. Journal of Clinical Investigation 100:1870–1881.

van der Flier A, Sonnenberg A. 2001. Function and interactions of integrins. Cell & Tissue Research 305:285–298.

Varner JA, Emerson DA, Juliano RL. 1995. Integrin alpha 5 beta 1 expression negatively regulates cell growth: Reversal by attachment to fibronectin. Molecular Biology of the Cell 6:725–740.

Watt FM. 2002. Role of integrins in regulating epidermal adhesion, growth and differentiation. EMBO Journal 21:3919–3926.

Weaver VM, Lelievre S, Lakins JN, Chrenek MA, Jones JC, Giancotti F, Werb Z, Bissell MJ. 2002. beta4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. Cancer Cell 2:205–216.

Wu AM, Senter PD. 2005. Arming antibodies: Prospects and challenges for immunoconjugates. Nature Biotechnology 23:1137–1146.

Yamada KM, Even-Ram S. 2002. Integrin regulation of growth factor receptors. Nature Cell Biology 4:E75–76.