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Minireview

Integrins: roles in cancer development and as treatment targets

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The integrin family of cell adhesion proteins promotes the attachment and migration of cells on the surrounding extracellular matrix (ECM). Through signals transduced upon integrin ligation by ECM proteins or immunoglobulin superfamily molecules, this family of proteins plays key roles in regulating tumour growth and metastasis as well as tumour angiogenesis. Several integrins play key roles in promoting tumour angiogenesis and tumour metastasis. Antagonists of several integrins ($\alpha5\beta1$, $\alpha v\beta3$ and $\alpha v\beta5$) are now under evaluation in clinical trials to determine their potential as therapeutics for cancer and other diseases.
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During the last 10 years, novel insights into the mechanisms that regulate cell survival as well as cell migration and invasion have led to the development of novel integrin-based therapeutics for the treatment of cancer. Several integrins play important roles in promoting cell proliferation, migration and survival *in vitro* and *in vivo*. Antagonists of these integrins suppress cell migration and invasion of primary and transformed cells and also induce apoptosis of primary cells. Integrin antagonists also block tumour angiogenesis and tumour metastasis. Currently, humanised antibody antagonists of integrins $\alpha5\beta1$ and $\alpha v\beta3$ as well as peptide inhibitors of integrins $\alpha v\beta3/\alpha v\beta5$ are under evaluation as angiogenesis-inhibiting therapeutics in cancer clinical trials.

INTEGRINS REGULATE CELL SURVIVAL AND MIGRATION

The invasion and survival of cells *in vivo* controls embryonic development, angiogenesis, tumour metastasis and other physiological processes (Aplin *et al*, 1998; Carmeliet and Jain, 2000; Hood and Cheresh, 2002). Cell surface receptors for the extracellular matrix (ECM), such as the integrins, play key roles in the regulation of normal and tumour cell migration and survival. The integrin family of cell adhesion proteins controls cell attachment to the ECM (Figure 1). While some integrins selectively recognise primarily a single ECM protein ligand (e.g., $\alpha5\beta1$ recognises primarily fibronectin), others can bind several ligands (e.g., integrin $\alpha v\beta3$ binds vitronectin, fibronectin, fibrinogen, denatured or proteolysed collagen, and other matrix proteins). Several integrins recognise the tripeptide Arg – Gly – Asp (e.g., $\alpha v\beta3$, $\alpha5\beta1$, $\alpha IIb\beta3$), whereas others recognise alternative short peptide sequences (e.g., integrin $\alpha4\beta1$ recognises EILDV and REDV in alternatively spliced CS-1 fibronectin). Inhibitors of integrin function include function-blocking monoclonal antibodies, peptide antagonists and small molecule peptide mimetics matrix (reviewed in Hynes, 1992; Cheresh, 1993).

Although integrins mediate cellular adhesion to ECM proteins found in intercellular spaces and basement membranes, they also transduce intracellular signals that promote cell migration (reviewed in Aplin *et al*, 1998; Schwartz and Shattil, 2000) as well as cell survival (Meredith *et al*, 1993; Stromblad and Cheresh, 1996). However, unlike growth factor receptors, integrins have no intrinsic enzymatic activity but activate signalling pathways strictly by coclustering with kinases and adaptor proteins in focal adhesion complexes. The association of integrins with polyvalent or crosslinked ECM proteins clusters integrins and their associated cofactors, thus activating integrin-regulated signalling pathways. For example, integrin ligation suppresses apoptosis by activating suppressors of apoptosis (Pankov *et al*, 2003) and by inhibiting caspase activation (Stupack *et al*, 2001; Kim *et al*, 2002). Integrins also stimulate cell migration by activating Rho and Rac GTPases (Ren *et al*, 1999) and by anchoring actin filaments to the membrane. These adhesion proteins promote cell cycle entry by stimulating expression of cyclins (Assoian and Schwartz, 2001). Integrin ligation, therefore, supports signal transduction cascades that promote cell proliferation, cell survival and cell migration. In contrast, inhibition of cell integrin – ligand interaction inhibits cell migration (Kim *et al*, 2000a, b; Bakre *et al*, 2002) and proliferation and induces apoptosis (Meredith *et al*, 1993; Boudreau *et al*, 1996; Stupack *et al*, 2001; Bakre *et al*, 2002; Kim *et al*, 2002).

INTEGRIN ROLES IN CELL SURVIVAL

Studies from several groups showed that cell attachment is required for the survival of normal cells (Meredith *et al*, 1993; Stupack *et al*, 2001; Bakre *et al*, 2002). Complete loss of cell contact with the substratum (e.g., suspension culture) or adhesion to a nonspecific substratum such as poly-L-lysine induces apoptosis ('anoikis') of primary cells such as fibroblasts (Meredith *et al*,

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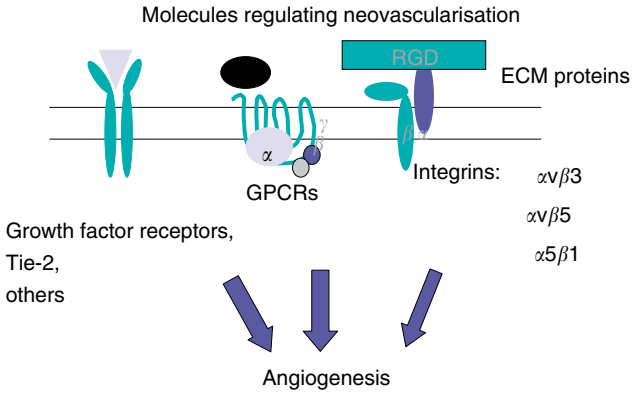

Figure 1 Molecules regulating angiogenesis. Growth factor receptors, other tyrosine kinase receptors such as Tie-2, G-protein-coupled receptors for angiogenesis modulating protein s such as interleukin-8 and parathyroid hormone-related peptide (Bakre *et al*, 2002), as well as integrins play key roles in the promotion of angiogenesis.

1993), endothelial cells (Bakre *et al*, 2002; Kim *et al*, 2002) and epithelial cells (Frisch and Francis, 1994; Boudreau *et al*, 1996; Stupack *et al*, 2001). In contrast, loss of contact with the substratum does not necessarily kill tumour cells. Anchorage-independent tumour cells survive loss of contact with the substrate because they accumulate mutational changes in survival factors, such as upregulation of Bcl 2 expression and/or loss of p53 activity, which render the cells independent of integrin-mediated survival signals.

Recent studies have shown that cell death can also occur when a subset of integrins in a cell fail to bind their ECM ligands (Stupack *et al*, 2001; Kim *et al*, 2002). For example, expression of $\alpha v\beta 3$ or $\alpha 5\beta 1$ can inhibit cell survival in cells attached to the matrix through other integrins (Stupack *et al*, 2001; Kim *et al*, 2002). The expression of $\alpha v\beta 3$ inhibits cell survival in cells attached to native collagen through integrin $\alpha 2\beta 1$ (Stupack *et al*, 2001). As integrin $\alpha v\beta 3$ does not bind native collagen, these results indicate that the unligated integrin $\alpha v\beta 3$ induces cell death. In a similar manner, inhibition of integrin $\alpha 5\beta 1$ activity with antibody antagonists induces apoptosis of endothelial cells that are attached to vitronectin through αv integrins (Kim *et al*, 2002). In addition, expression of dominant negative integrins (e.g., Tac-$\beta 3$, the IL-2 receptor fused with the integrin beta 3 subunit cytoplasmic tail) also inhibits survival by impairing normal integrin-mediated survival signalling (Stupack *et al*, 2001). Integrin ligation suppresses caspase 8 activation, while unligated integrins facilitate caspase 8 activation in a stress response and death receptor independent manner (Stupack *et al*, 2001; Kim *et al*, 2002). Additional studies suggest that unligated integrins activate membrane-associated protein kinase A (PKA), which itself can activate caspase 8 in endothelial cells (Kim *et al*, 2002). Thus, in normal cells, some integrins regulate survival when ligated and induce apoptosis when unligated.

INTEGRIN ROLES IN CELL MIGRATION

While integrin ligation by the ECM positively regulates migration, antagonising integrins inhibits cell migration. Although blocking integrin ligation can prevent cell attachment to the ECM and thus inhibit migration, recent studies show that antagonised integrins actively inhibit signal transduction leading to cell migration (Kim *et al*, 2000b). For example, the inhibition of integrin $\alpha 5\beta 1$ negatively regulates fibroblast, endothelial cell and tumour cell migration even when other integrin receptors for provisional matrix proteins are ligated (Kim *et al*, 2000b). Antagonists of integrin $\alpha 5\beta 1$ suppress cell migration on vitronectin, but not cell attachment to vitronectin, indicating that these antagonists affect the migration machinery rather than integrin receptors for vitronectin (Kim *et al*, 2000b). In fact, $\alpha 5\beta 1$ antagonists activate PKA, which then inhibits cell migration by disrupting the formation of stress fibres (Kim *et al*, 2000b). Direct activation of PKA by forskolin or by overexpression of the catalytic, active subunit of PKA also inhibits cell migration (Bakre *et al*, 2002; Kim *et al*, 2000b). Thus, integrins regulate cell migration by making contact with the substratum and by promoting signal transduction cascades that support migration.

INTEGRINS REGULATE ANGIOGENESIS

Angiogenesis is the process by which new blood vessels develop from pre-existing vessels. The growth of new blood vessels promotes embryonic development, wound healing and the female reproductive cycle, and also plays a key role in the pathological development of solid tumour cancers, haemangiomas, diabetic retinopathy, age-related macular degeneration, psoriasis, gingivitis, rheumatoid arthritis and possibly osteoarthritis and inflammatory bowel disease (reviewed in Carmeliet and Jain, 2000). New advances in understanding the mechanisms regulating angiogenesis, such as those that promote cell migration and invasion, are leading to the development of novel therapeutics for cancer.

Growth factors released by hypoxic tissues or pathological tissues such as tumours stimulate new blood vessel growth. New vessels grow by sprouting from pre-existing vessels (reviewed in Carmeliet and Jain, 2000) or by recruitment of bone marrow-derived endothelial progenitor cells (Asahara *et al*, 1997). While growth factors and their receptors play key roles in angiogenic sprouting, adhesion to the ECM also regulates angiogenesis (Figure 2). Adhesion promotes endothelial cell survival (Kim *et al*, 2002; Stupack and Cheresh, 2002), as well as endothelial cell proliferation and motility (Kim *et al*, 2000a, b) during new blood vessel growth. One ECM protein in particular, fibronectin, is associated with vascular proliferation (Kim *et al*, 2000a, b); it is expressed in provisional vascular matrices and provides proliferative signals to vascular cells during wound healing, atherosclerosis and hypertension. Notably, fibronectin-null mice die early in development from a collection of defects, which include an

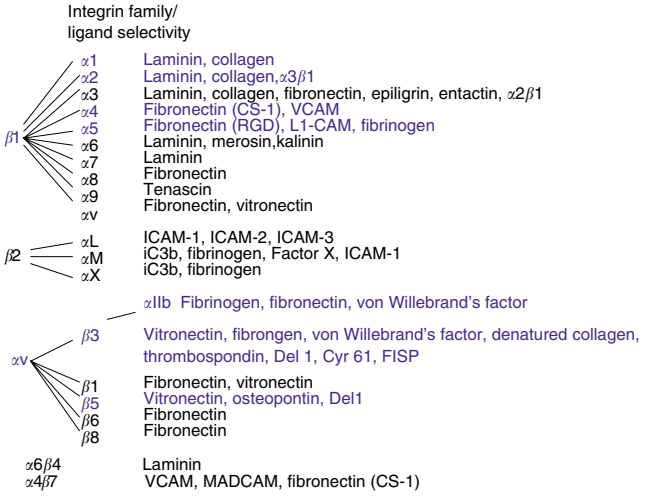

Figure 2 Integrin family. Integrin alpha beta heterodimers can be grouped into three subfamilies. Integrins $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha 4\beta 1$, $\alpha v\beta 3$ and $\alpha v\beta 5$ (highlighted in blue) have been shown to play important roles in regulating tumour angiogenesis.

improperly formed vasculature (George *et al*, 1993, 1997). Recent experimental studies showed that fibronectin regulates angiogenesis, as antibody inhibitors of fibronectin block angiogenesis (Kim *et al*, 2000a).

Studies in experimental angiogenesis models and in mutant mice indicate that several integrins play key roles in regulating angiogenesis. Embryonic deletion of integrin $\alpha5\beta1$ induces early mesenchymal abnormalities, which include defects in the organisation of the emerging vasculature (Yang *et al*, 1993; Goh *et al*, 1997) and defects in the ability of endothelial cells to form vessel-like structures *ex vivo* (Taverna and Hynes, 2001; Francis *et al*, 2002). Similarly, loss of integrin $\alpha4\beta1$ leads to aorta, heart and other vascular malformations (Yang *et al*, 1995). Deletion of the αv subunit causes 80% of embryos to die early in development from uncertain causes, while the few surviving embryos die a few hours after birth with significant defects in brain development, including failure of blood vessels to form properly (Bader *et al*, 1998). In contrast, individual loss of the $\beta3$or$\beta5$ subunit during embryogenesis does not cause noticeable defects in the formation of the cardiovascular system (Hodivala-Dilke *et al*, 1999; Huang *et al*, 2000). In fact, one study show that loss of the $\beta3$ or the $\beta3$ and $\beta5$ subunits promotes tumour angiogenesis (Reynolds *et al*, 2002). These studies led to the controversial conclusion that the αvβ3/αvβ5 integrins are not required for angiogenesis, but instead may suppress angiogenesis. As many studies have shown that $\alpha v\beta3$ and $\alpha v\beta5$ inhibitors block angiogenesis by inducing apoptosis in proliferating endothelial cells (Brooks *et al*, 1994b), it is possible that loss of the integrin-mediated death mechanism can lead to enhanced angiogenesis (Cheresh and Stupack, 2002). In fact, loss of either the $\beta3$ or $\beta5$ subunits does block angiogenesis induced by the angiomatrix protein Del-1 (Zhong *et al*, 2003). Thus, integrins appear to have diverse roles in the establishment of the cardiovascular system, with integrin $\alpha5\beta1$ clearly playing a major role during development of the vascular system.

Studies in experimental models of angiogenesis also indicate that several integrins can play important roles in regulating angiogenesis in normal animals. The expression of both integrins $\alpha v\beta3$ (Brooks *et al*, 1994a) and $\alpha5\beta1$ (Kim *et al*, 2000a) are significantly upregulated on endothelium during angiogenesis. The expression of integrins $\alpha v\beta3$ and $\alpha5\beta1$ partially controls angiogenesis; neither integrin is expressed by quiescent endothelium and both are expressed in response to angiogenic growth factors (Brooks *et al*, 1994a; Kim *et al*, 2000a, b). Their expression is controlled by the transcription factor Hox D3 (Boudreau *et al*, 1997; Boudreau and Varner, 2003; Zhong *et al*, 2003). Hox D3 is a Homeobox gene expressed by ECs that may regulate an angiogenic switch. When expressed *in vivo*, Hox D3 promotes a haemangioma-like proliferation of blood vessels (Boudreau *et al*, 1997; Zhong *et al*, 2003); this transcription factor promotes the expression of integrin $\alpha v\beta3$, $\alpha5\beta1$ and uPA, molecules with established roles in angiogenesis. Thus, Hox D3 may provide a switch to activate a program of angiogenesis. Once integrins $\alpha5\beta1$ and $\alpha v\beta3$ are expressed, angiogenesis depends on each integrin as antagonists of each can block angiogenesis *in vivo* (Brooks *et al*, 1994a, b; Kim *et al*, 2000a). Antibody and peptide antagonists of integrins $\alpha v\beta3$ and $\alpha5\beta1$ inhibit growth factor as well as tumour angiogenesis, tumour growth and tumour metastasis (Brooks *et al*, 1994a, b; Carron *et al*, 1998; Kim *et al*, 2000a; Stoeltzing *et al*, 2003). These studies indicate that these integrins function in part by promoting survival in proliferating endothelial cells *in vivo* (Brooks *et al*, 1994b; Kim *et al*, 2002). Studies of the signals transduced when integrins are antagonised indicate that unligated integrins activate PKA, which then activates caspase 8 and induces apoptosis (Bakre *et al*, 2002; Kim *et al*, 2002).

In addition, other integrins have been shown to regulate angiogenesis. Integrin $\alpha v\beta5$ promotes VEGF-, but not bFGF-, mediated angiogenesis (Friedlander *et al*, 1995). Integrin receptors for laminin and collagen also play roles in regulating blood vessel formation as antagonists of $\alpha2\beta1$ and $\alpha1\beta1$ suppressed VEGF-mediated angiogenesis (Senger *et al*, 1997). Thus, integrins play key roles in regulating tumour angiogenesis, and integrin antagonists hold promise as future therapeutics for cancer.

INTEGRINS PLAY ROLES IN TUMOUR INVASION AND METASTASIS

Tumour metastasis promotes the spread of tumours to local and distant sites away from primary tumours. Metastasis is the leading cause of the morbidity and mortality associated with cancer. Tumour cells isolated from metastases are highly migratory and invasive. Therefore, understanding the mechanisms regulating cell migration may be helpful in developing new modes of therapy for metastatic cancer.

Increased levels of expression of integrins $\alpha v\beta3$ is closely associated with increased cell invasion and metastasis (Felding-Habermann *et al*, 2002). Notably, integrin $\alpha v\beta3$ is expressed on invasive melanoma but not benign nevi or normal melanocytes (Gehlsen *et al*, 1992). Additionally, increased $\alpha v\beta3$ expression levels correlate with increased rates of melanoma metastases (Nip *et al*, 1992).

Integrin $\alpha6$ expression is also significantly upregulated in numerous carcinomas, including head and neck cancers and breast cancer (Garzino-Demo *et al*, 1998; Mercurio *et al*, 2001; Ramos *et al*, 2002). Integrin $\alpha6\beta4$ expression enhances tumour cell invasiveness and metastasis, particularly in breast carcinomas (Mercurio *et al*, 2001; Ramos *et al*, 2002). Thus, antagonists of these integrins may be useful to prevent the spread of tumour cells.

INTEGRIN INHIBITORS AS THERAPEUTIC AGENTS FOR CANCER

Several integrin inhibitors are currently under investigation as therapeutics for cancer. Antibody and peptide inhibitors of integrins $\alpha v\beta3$ and $\alpha v\beta5$ (for review, see Kerr *et al*, 2002) and of $\alpha5\beta1$ are currently in clinical trials for the inhibition of angiogenesis in cancer. A humanised anti-$\alpha v\beta3$ antibody, Vitaxin, is currently in Phase II trials for cancer (Gutheil *et al*, 2000; Patel *et al*, 2001; Posey *et al*, 2001; Mikecz, 2000), while a humanised anti-$\alpha5\beta1$ antibody is in Phase I trials for cancer (Varner, personal communication; www.pdl.com). A cyclic peptide inhibitor of integrin $\alpha v\beta3/\alpha v\beta5$, Cilengitide, is in Phase I/II trials for glioblastoma and other cancers (Burke *et al*, 2002; Eskens *et al*, 2003; Smith, 2003). Other promising integrin $\alpha5\beta1$- and $\alpha v\beta3$-blocking peptides with antitumour angiogenesis and tumour metastasis activities are currently in preclinical development (Carron *et al*, 1998; Reinmuth *et al*, 2003; Stoeltzing *et al*, 2003). As Avastin, the antibody inhibitor of VEGF, has recently shown promise as a therapeutic for colon cancer in Phase III clinical trials (Fernando and Hurwitz, 2003), these integrin-based antiangiogenesis therapeutics hold great promise as powerful therapeutics for the treatment of cancer.

CONCLUSION

The studies reviewed here indicate that integrin promote cellular migration and survival in tumour and primary cells. Antagonists of integrins $\alpha v\beta3$, $\alpha5\beta1$, $\alpha v\beta5$ and $\alpha6\beta4$ show great promise as potential inhibitors of tumour growth and metastasis as well as tumour angiogenesis. Clinical trials are currently underway to evaluate inhibitors of integrin $\alpha v\beta3$, $\alpha v\beta5$ and $\alpha5\beta1$ for their usefulness in the treatment of cancer.

REFERENCES

Aplin AE, Howe A, Alahari SK, Juliano RL (1998) Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobulin-cell adhesion molecules and selectins. *Pharmacol Rev* **50:** 197 – 263

Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* **275:** 964 – 967

Assoian RK, Schwartz MA (2001) Coordinate signaling by integrins and receptor tyrosine kinases in the regulation of G1 phase cell-cycle progression. *Curr Opin Genet Dev* **11:** 48 – 53

Bader BL, Rayburn H, Crowley D, Hynes RO (1998) Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. *Cell* **95:** 507 – 519

Bakre MM, Zhu Y, Yin H, Burton DW, Terkeltaub R, Deftos LJ, Varner JA. (2002) Parathyroid hormone-related peptide is a naturally occurring, protein kinase A-dependent angiogenesis inhibitor. *Nat Med* **8:** 995 – 1003

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

Boudreau N, Andrews C, Srebow A, Ravanpay A, Cheresh DA (1997) Induction of the angiogenic phenotype by Hox D3. *J Cell Biol* **139:** 257 – 264

Boudreau N, Varner J (2003) The homeobox transcription factor Hox D3 promotes integrin $\alpha5\beta1$ expression and function during angiogenesis. *J Biol Chem*, in press.

Boudreau N, Werb Z, Bissell MJ (1996) Suppression of apoptosis by basement membrane requires three-dimensional tissue organization and withdrawal from the cell cycle. *Proc Natl Acad Sci USA* **93:** 1353 – 3509

Burke PA, DeNardo SJ, Miers LA, Lamborn KR, Matzku S, DeNardo GL (2002) Cilengitide targeting of alpha(v)beta(3) integrin receptor synergizes with radioimmunotherapy to increase efficacy and apoptosis in breast cancer xenografts. *Cancer Res* **62:** 4263 – 4272

Carmeliet P, Jain RK (2000) Angiogenesis in cancer and disease. *Nature* **407:** 249 – 257

Carron CP, Meyer DM, Pegg JA, Engleman VW, Nickols MA, Settle SL, Westlin WF, Ruminski PG, Nichols GA (1998) A peptidomimetic antagonist of the integrin $\alpha v\beta3$ inhibits Leydig cell tumor growth and development of hypercalcemia of malignancy. *Cancer Res* **58:** 1930 – 1955

Cheresh D (1993) Integrins: structure, function and biological properties. *Adv Mol Cell Biol* **6:** 225 – 252

Cheresh DA, Stupack DG (2002) Integrin-mediated death: an explanation of the integrin-knockout phenotype? *Nat Med* **8:** 193 – 194

Eskens FA, Dumez H, Hoekstra R, Perschl A, Brindley C, Bottcher S, Wynendaele W, Drevs J, Verweij J, van Oosterom AT 2003)) Phase I and pharmacokinetic study of continuous twice weekly intravenous administration of Cilengitide (EMD 121974), a novel inhibitor of the integrins alphavbeta3 and alphavbeta5 in patients with advanced solid tumours. *Eur J Cancer* **39:** 917 – 926

Felding-Habermann B, Fransvea E, O'toole TE, Manzuk L, Faha B, Hensler M (2002) Involvement of tumor cell integrin alpha v beta 3 in hematogenous metastasis of human melanoma cells. *Clin Exp Metast* **19:** 427 – 436

Fernando NH, Hurwitz HI (2003) Inhibition of vascular endothelial growth factor in the treatment of colorectal cancer. *Semin Oncol* **30:** 39 – 50

Francis SE, Goh KL, Hodivala-Dilke K, Bader BL, Stark M, Davidson D, Hynes RO (2002) Central roles of alpha5beta1 integrin and fibronectin in vascular development in mouse embryos and embryoid bodies. *Arterioscler Thromb Vasc Biol* **22:** 927 – 933

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, Francis H (1994) Disruption of epithelial cell – matrix interactions induces apoptosis. *J Cell Biol* **124:** 619 – 626

Garzino-Demo P, Carrozzo M, Trusolino L, Savoia P, Gandolfo S, Marchisio PC (1998) Altered expression of alpha 6 integrin subunit in oral squamous cell carcinoma and oral potentially malignant lesions. *Oral Oncol* **34:** 204 – 210

Gehlsen KR, Davis GE, Sriramarao P (1992) Integrin expression in human melanoma cells with differing invasive and metastatic properties. *Clin Exp Metast* **10:** 111 – 120

George EL, Baldwin HS, Hynes RO (1997) Fibronectins are essential for heart and blood vessel morphogenesis but are dispensable for initial specification of precursor cells. *Blood* **90:** 3073 – 3081

George EL, Georges-Labouesse EN, Patel-King RS, Rayburn H, Hynes RO (1993) Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. *Development* **119:** 1079 – 1091

Goh KL, Yang JT, Hynes RO (1997) Mesodermal defects and cranial neural crest apoptosis in $\alpha5$ integrin-null embryos. *Development* **124:** 4309 – 4319

Gutheil JC, Campbell TN, Pierce PR, Watkins JD, Huse WD, Bodkin DJ, Cheresh DA (2000) Targeted antiangiogenic therapy for cancer using vitaxin: a humanized monoclonal antibody to the integrin alphavbeta3. *Clin Cancer Res* **6:** 3056 – 3061

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. *J Clin Invest* **103:** 229 – 238

Hood J, Cheresh D (2002) Role of integrins in cell invasion and migration. *Nat Rev* **2:** 91 – 103

Huang X, Griffiths M, Wu J, Farese Jr. RV, Sheppard D (2000) Normal development, wound healing, and adenovirus susceptibility in beta5-deficient mice. *Mol Cell Biol* **20:** 755 – 759

Hynes RO (1992) Integrins: versatility, modulation and signaling in cell adhesion. *Cell* **69:** 11 – 25

Kerr JS, Slee AM, Mousa SA (2002) The alpha v integrin antagonists as novel anticancer agents: an update. *Expert Opin Investig Drugs* **11:** 1765 – 1774

Kim S, Bakre M, Yin H, Varner J (2002) Inhibition of endothelial cell survival and angiogenesis by protein kinase A. *J Clin Invest* **110:** 933 – 941

Kim S, Bell K, Mousa SA, Varner J (2000a) Regulation of angiogenesis *in vivo* by ligation of integrin $\alpha5\beta1$ with the central cell binding domain of fibronectin. *Am J Path* **156:** 1345 – 1362

Kim S, Harris M, Varner JA (2000b) Regulation of integrin $\alpha v\beta3$-mediated endothelial cell migration and angiogenesis by integrin $\alpha5\beta1$ and protein kinase A. *J Biol Chem* **275:** 33920 – 33928

Mercurio AM, Bachelder RE, Chung J, O'Connor KL, Rabinovitz I, Shaw LM, Tani T (2001) Integrin laminin receptors and breast carcinoma progression. *J Mammary Gland Biol Neoplasia* **6:** 299 – 309

Meredith Jr. JE, Fazeli B, Schwartz MA (1993) The extracellular matrix as a cell survival factor. *Mol Biol Cell* **4:** 953 – 961

Mikecz K (2000) Vitaxin. *Curr Opin Invest Drugs* **1:** 199 – 203

Nip J, Shibata H, Loskutoff DJ, Cheresh DA, Brodt P (1992) Human melanoma cells derived from lymphatic metastases use integrin alpha v beta 3 to adhere to lymph node vitronectin. *J Clin Invest* **90:** 1406 – 1413

Patel SR, Jenkins J, Papadopolous N, Burgess MA, Plager C, Gutterman J, Benjamin RS. (2001) Pilot study of vitaxin – an angiogenesis inhibitor – in patients with advanced leiomyosarcomas. *Cancer* **92:** 1347 – 1348

Pankov R, Cukierman E, Clark K, Matsumoto K, Hahn C, Poulin B, Yamada KM (2003) Specific beta1 integrin site selectively regulates Akt/protein kinase B signaling via local activation of protein phosphatase 2A. *J Biol Chem* **278:** 18671 – 18681

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 vbeta 3) antibody in patients with metastatic cancer. *Cancer Biother Radiopharm* **16:** 125 – 132

Ramos DM, But M, Regezi J, Schmidt BL, Atakilit A, Dang D, Ellis D, Jordan R, Li X (2002) Expression of integrin beta 6 enhances invasive behavior in oral squamous cell carcinoma. *Matrix Biol* **21:** 297 – 307

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 Res* **63:** 2079 – 2087

Ren X-D, Kiosses WB, Schwartz MA (1999) Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO J.* **18:** 578 – 585

Reynolds LE, Wyder L, Lively JC, Taverna D, Robinson SD, Huang X, Sheppard D, Hynes RO, Hodivala-Dilke KM (2002) Enhanced pathological angiogenesis in mice lacking $\beta3$ and $\beta5$integrins. *Nat Med* **8:** 229 – 238

Schwartz MA, Shattil SJ (2000) Signaling networks linking integrins and Rho family GTPases. *Tips* **25:** 388 – 391

Senger DR, Claffey KP, Benes JE, Perruzzi CA, Sergiou AP, Detmar M (1997) Angiogenesis promoted by vascular endothelial growth factor: regulation through alpha1beta1 and alpha2beta1 integrins. *Proc Natl Acad Sci USA* **94:** 13612 – 13617

Smith JW (2003) Cilengitide Merck. *Curr Opin Investig Drugs* **4:** 741 – 745

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. *Int J Cancer* **20:** 496 – 503

Stromblad S, Becker JC, Yebra M, Brooks PC, Cheresh DA (1996) Suppression of p53 activity and p21WAF1/CIP1 expression by vascular cell integrin alphaVbeta3 during angiogenesis. *J Clin Invest* **98:** 426 – 433

Stupack DG, Puente XS, Butsaboualoy S, Storgard CM, Cheresh DA (2001) Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. *J Cell Biol* **155:** 459 – 470

Taverna D, Hynes RO (2001) Reduced blood vessel formation and tumor growth in alpha 5-integrin-negative teratocarcinomas and embryoid bodies. *Cancer Res* **61:** 5255 – 5261

Yang JT, Rayburn H, Hynes RO (1993) Embryonic mesodermal defects in α5 integrin-deficient mice. *Development* **119:** 1093 – 1105

Yang JT, Rayburn H, Hynes RO (1995) Cell adhesion events mediated by alpha 4 integrins are essential in placental and cardiac development. *Development* **121:** 549 – 560

Zhong J, Eliceiri B, Stupack D, Penta K, Sakamoto G, Hynes R, Coleman M, Quertermous T, Boudreau N, Varner J (2003) Neovascularization of ischemic tissues by gene delivery of the extracellular matrix protein Del1. *J Clin Invest* **112:** 30 – 41