Integrins: roles in cancer development and as treatment targets

H Jin¹ and J Varner*¹,²
¹John and Rebecca Moores Comprehensive Cancer Center, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0912, USA; ²Department of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0912, USA

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 (α5β1, αvβ3 and αvβ5) are now under evaluation in clinical trials to determine their potential as therapeutics for cancer and other diseases.

British Journal of Cancer (2004) 90, 561 – 565. doi:10.1038/sj.bjc.6601576 www.bjcancer.com © 2004 Cancer Research UK

Keywords: angiogenesis; metastasis; apoptosis; integrin α5β1; integrin αvβ3

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., α5β1 recognises primarily fibronectin), others can bind several ligands (e.g., integrin αvβ3 binds vitronectin, fibronectin, fibrinogen, denatured or proteolyzed collagen, and other matrix proteins). Several integrins recognise the tripeptide Arg–Gly–Asp (e.g., αvβ3, α5β1, α1β3), whereas others recognise alternative short peptide sequences (e.g., integrin α4β1 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; Schwarz 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 cofluctering with kinases and adapter 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,
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, ginvigritis, 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
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 α5β1 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 αvβ3 leads to aorta, heart and other vascular malformations (Yang et al., 1995). Deletion of the αv subunit also results in embryos to die at 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 α3 or β5 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 β3 or the β3 and β5 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 αvβ3 and αvβ5 inhibitors block angiogenesis by inducing apoptosis in proliferating endothelial cells (Brooks et al., 1994b), it is possible that loss of these integrin-mediated death mechanism can lead to enhanced angiogenesis (Cheresh and Stupack, 2002). In fact, loss of either the β3 or β5 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 α5β1 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 αvβ3 (Brooks et al., 1994a) and α5β1 (Kim et al., 2000a) are significantly upregulated on endothelium during angiogenesis. The expression of integrins αvβ3 and α5β1 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 haemangiomalike proliferation of blood vessels (Boudreau et al., 1997; Zhong et al., 2003); this transcription factor promotes the expression of integrin αvβ3, α5β1 and uPA, molecules with established roles in angiogenesis. Thus, Hox D3 may provide a switch to activate a program of angiogenesis. Once integrins α5β1 and αvβ3 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 αvβ3 and α5β1 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 αvβ5 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 α2β1 and α1β1 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, and 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 αvβ3 is closely associated with increased cell invasion and metastasis (Felding-Habermann et al., 2002). Notably, integrin αvβ3 is expressed on invasive melanoma but not benign nevi or normal melanocytes (Gehlsen et al., 1992). Additionally, increased αvβ3 expression levels correlate with increased rates of melanoma metastases (Nip et al., 1992).

Integrin α6 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 α6β4 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 αvβ3 and αvβ5 (for review, see Kerr et al., 2002) and of α5β1 are currently in clinical trials for the inhibition of angiogenesis in cancer. A humanised anti-αvβ3 antibody, Vitaxin, is currently in Phase II trials for cancer (Guthell et al., 2000; Patel et al., 2001; Posey et al., 2001; Miecz, 2000), while a humanised anti-α5β1 antibody is in Phase I trials for cancer (Varner, personal communication; www.pdl.com). A cyclic peptide inhibitor of integrin αvβ3/αvβ5, Cilengtide, is in Phase I/II trials for glioblastoma and other cancers (Burke et al., 2002; Eskens et al., 2003; Smith, 2003). Other promising integrin α5β1- and αvβ3-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 αvβ3, α5β1, αvβ5 and α6β4 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 αvβ3, αvβ5 and α5β1 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: 1–263

Asoin 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, Terkelhaub R, Deftos LJ, Varner JA, Varner J (2002) Parathryoid 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 alpha v beta 3 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: 1353–1359

Burke PA, DeNardo SJ, Miers LA, Lamborn KR, Matzku S, DeNardo GL (2002) Ciligentide targeting of alpha(v)beta(3) integrin receptor synergizes with radiomunotherapy 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, Ruminuki PG, Nichols GA (1998) A peptidomimetic antagonist of the integrin alpha v beta 3 inhibits Leydig cell tumor growth and development of hypercalcemia of malignancy. Cancer Res 58: 1930–1935

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

Ekens FA, Dumez H, Hoeckstra 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 alpha v beta 3 and beta 5 to adhere to lung cancer cells. J Clin Cancer Res 9: 1345–1362

Felding-Haberemann B, Fransvea E, O’toole TE, Manzuk L, Faha B, Henders 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 alpha3beta 1 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, Kincade 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, Srimarao P (1992) Integrin expression in human melanoma cells with differing invasive and metastatic properties. Clin Exp Metast 10: 111–120

George EL, Baldwin HS, Nemes 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 55 integrin-null embryos. Development 124: 4309–4319

Guthie 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) Beta 3-Integran-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 adenosine 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, See A, Assoian RK, Maus SA (2002) The alpha v integrin antagonists as novel anticancer agents: an update. Expert Opin Invest Drugs 11: 1765–1774

Kim S, Bakre M, Yin H, Varner JA (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 alpha v beta 3 with the central cell binding domain of fibronectin. Am J Pathol 156: 1345–1356

Kim S, Harris M, Varner JA (2000b) Regulation of integrin alpha vbeta 3-mediated endothelial cell migration and angiogenesis by integrin alpha 5beta 1 and protein kinase A. J Biol Chem 275: 33920–33928

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

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

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

Nip J, Shibata H, Loskutoff D, 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, Papadopoulos N, Burgess MA, Plager C, Gutterman J, Mikecz K (2000) Vitaxin. Curr Opin Invest Drugs 1: 199–203

Patel SR, Jenkins J, Papadopoulos N, Burgess MA, Plager C, Gutterman J, Mikecz K (2000) Vitaxin. Curr Opin Invest Drugs 1: 199–203

Ramos DM, But M, Regezi J, Schmidt BL, Atakilit A, Dang D, Ellis D, Jordan KM (2003) Specific beta 1 integrin site selectively regulates Akt/protein kinase B signaling via local activation of protein phosphatase 2A. J Biol Chem 278: 18671–18681

Posey JA, Khazaie MB, DeGrossro 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 Bitherapy Radiopharm 16: 125–132

Ramos DM, But M, Regezi J, Schmidt BL, Atakilit A, Dang D, Ellis D, Jordan KM (2003) Specific beta 1 integrin site selectively regulates Akt/protein kinase B signaling via local activation of protein phosphatase 2A. J Biol Chem 278: 18671–18681

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 beta 5 and beta 3 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
Tavera 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