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Integrins in angiogenesis and lymphangiogenesis

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Preface

Blood vessels promote tumor growth, while both blood and lymphatic vessels facilitate tumor metastasis by serving as conduits for the transport of tumor cells to new sites. Angiogenesis and lymphangiogenesis are regulated by integrins, which are members of a family of cell surface receptors whose ligands are extracellular matrix proteins and immunoglobulin superfamily molecules. Select integrins promote endothelial cell migration and survival during angiogenesis and lymphangiogenesis, while other integrins promote pro-angiogenic macrophage trafficking to tumors. Several integrin-targeted therapeutic agents are currently in clinical trials for cancer therapy. Here we review the evidence implicating integrins as a family of fundamental regulators of angiogenesis and lymphangiogenesis.

Introduction

Angiogenesis, the development of new blood vessels, is a fundamental physiological process that promotes embryonic development, tissue repair and fertility, yet that also promotes chronic inflammation, tumor growth and tumor metastasis[1]. New blood vessels in tumors can grow by sprouting from preexisting vessels or by recruitment of rare, circulating bone marrow-derived endothelial progenitor cells[1-3]. Tumor cells, macrophages, and fibroblasts within tumors can secrete factors, such as vascular endothelial growth factor (VEGF), that induce blood vessel growth in tumors[2,4-5]. Basic and clinical studies indicate that suppression of angiogenesis can inhibit tumor progression and metastasis[6]. Many lines of investigation implicate integrins, which are key regulators of endothelial cell migration and survival, as key regulators of tumor angiogenesis (Figure 1).

Like angiogenesis, lymphangiogenesis - the growth of new lymphatic vessels - promotes tumor metastasis[2,7-9]. The lymphatic system is comprised of a network of blind-ended, thin walled lymphatic capillaries, collecting vessels and specialized secondary immune organs, including lymph nodes, tonsils, **Peyer's Patches [G]** and the spleen. Lymphatic vessels drain protein-rich interstitial fluids and immune cells through the lymph nodes and return fluids back to the circulation at the thoracic duct. After entering lymph nodes, antigen-presenting cells such as dendritic cells may activate B- and T-cell immune responses. Tumors induce the growth of new lymphatic vessels within tumors and draining lymph nodes, enhancing dendritic cell trafficking to lymph nodes; increased lymphatic vessel density in tumors is also associated with increased metastasis to lymph nodes[7-9]. New findings indicate that select integrins can modulate lymphangiogenesis and may thereby affect tumor metastasis (Figure 1).

Angiogenesis and lymphangiogenesis can be stimulated by tumor-associated macrophages. Circulating bone marrow-derived cells (monocytes) migrate into tumors in response to tumor-secreted chemokines and differentiate into macrophages. Pro-angiogenic tumor macrophages

-Links to web sites:
http://www.cancerpublications.com/newsletter/colorectal/AIO/v1n4/Bukowski/index.htm Ronald M. Bukowsi: Integrins and inhibitors of angiogenesis

release a number of potent pro-angiogenic cytokines, such as VEGF-A, VEGF-C, tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8) and basic fibroblast growth factor (bFGF) [4-5] and express a broad array of extracellular matrix degrading proteases, including urokinase-type plasminogen activator (uPA), the matrix metalloproteinases MMP-2, MMP-7, MMP-9 and MMP12 and elastase[4]. Importantly, new evidence suggests that macrophage infiltration can activate the **angiogenic switch [G]** in spontaneous tumors[5]. Select integrins play key roles in regulating the trafficking of circulating monocytes and progenitor cells to tumors.

The Integrin Family of Adhesion Receptors

The integrin family is an extensive group of structurally related receptors for extracellular matrix (ECM) proteins and immunoglobulin superfamily molecules. Integrins are divalent cation-dependent heterodimeric membrane glycoproteins comprised of non-covalently associated α and β subunits that promote cell attachment and migration on the surrounding extracellular matrix. Eighteen α and eight β subunits can associate to form twenty-four unique integrin heterodimers. Each integrin subunit consists of an extracellular domain, a single transmembrane region, and a short (approximately thirty to forty amino acids) cytoplasmic region[10]. While some integrins, such as α5β1, primarily recognize a single ligand, others, such as αvβ3 can bind several ligands. Many integrins, including αvβ3, α5β1, αIIbβ3, αvβ6, and α3β1 recognize the tripeptide Arg-Gly-Asp (RGD) in their ligands[11]. Sequences flanking the RGD peptide are also important for integrin selectivity[12]. Other integrins recognize alternative short peptide sequences; for example, integrin α4β1 recognizes Glu-Ile-Leu-Asp-Val (EILDV) and Arg-Glu-Asp-Val (REDV) in the alternatively spliced fibronectin domain known as IIICS[13]. Some integrins, such as α4β1, can also bind cell surface receptors, such as Vascular Cell Adhesion Molecule-1 (VCAM-1), to promote cell-cell adhesion (Text Box 1).[14]

Integrin ligation promotes integrin clustering and subsequent integrin-mediated intracellular signal transduction. Unlike growth factor receptors, integrins have no intrinsic enzymatic or kinase activities, but activate complex signaling pathways by coclustering with kinases and adaptor proteins in focal adhesion complexes. A number of signaling moieties are activated by integrins and many of these are found within focal adhesion complexes. Focal adhesion complexes are comprised of integrins, protein kinases - such as focal adhesion kinase (FAK) and Src - adaptor proteins such as Shc, signaling intermediates such as Rho family GTPases, actin binding cytoskeletal proteins such as talin, α-actinin, paxillin, tensin and vinculin[15-16] and other signaling proteins. Integrin signaling promotes cell migration, proliferation and survival (Text Box 2). Loss of integrin ligation inhibits these events and unligated integrins can actively initiate apoptosis, even without loss of cell attachment. This form of death is stress response- and death-receptor-independent, but caspase 8-dependent, and has been called 'integrin mediated death'[17].

In the last several years, some of the key molecular mechanisms that regulate angiogenesis and lymphangiogenesis have been delineated. Many lines of investigation implicate integrins as key regulators of endothelial cell migration and survival during these events. In this Review, we will discuss the evidence that supports roles for various integrins in the regulation of angiogenesis and lymphangiogenesis and we discuss the status of integrin-based therapeutics for the treatment of cancer.

Integrins in Vascular Endothelium and Angiogenesis

In vitro and *in vivo* data have implicated a number of endothelial cell integrins in the regulation of cell growth, survival and migration during angiogenesis. These integrins include the heterodimers α1β1, α2β1, α4β1, α5β1, α6β1, α6β4, α9β1, αvβ3 and αvβ5, (Table 1). Additionally, the glial cell integrin αvβ8 regulates brain blood vessel development.[18] Distinct experimental approaches, such as inhibition of angiogenesis by antagonists, knockin mutations

and genetic deletions in ovo have led to conflicting theories of the roles of several integrins in angiogenesis, including αv integrins and α2β1 integrin. However, increasing molecular evidence *in vivo* points to major roles key integrins in tumor angiogenesis.

Alpha v Integrins

The αv integrin subunit can heterodimerize with several different beta subunits (β1, β3, β5, β6 and β8) to achieve unique ligand-binding profiles. The integrin αvβ3, a receptor for RGD-containing proteins such as vitronectin, fibronectin, fibrinogen and osteopontin (which are components of the ECM), was the first of the alpha v integrins to be characterized[19]. Integrin αvβ3 was the first αv integrin to be shown to regulate angiogenesis.[20] This integrin is widely expressed on blood vessels in human tumor biopsies but not on vessels in normal human tissues. Its expression on endothelial cells is stimulated by angiogenic growth factors such as bFGF, TNFα, and IL-8 and it is upregulated on endothelium in tumors, wounds and sites of inflammation[20]. While angiogenesis during wound healing is tightly regulated and self-limiting, angiogenesis associated with chronic inflammation and cancer is often persistent and abnormal[1]. However, many of the same molecules that regulate angiogenesis in wound healing, such as integrin αvβ3, also regulate pathological angiogenesis. Increasing evidence points to important causative links between inflammation and cancer and some evidence suggests that inflammation promotes the **angiogenic switch** [G] in tumors[4-5] (Textbox 3).

Several experimental approaches indicate that αvβ3 plays a key role in endothelial cell survival and migration during angiogenesis[20-21]. Integrin αvβ3 is expressed in response to angiogenic growth factors and tumors in **chick chorioallantoic membrane** [G], mouse, rabbit and human models of angiogenesis and tumor growth[20-23]. Antagonists of αvβ3 inhibited angiogenesis and tumor growth in a variety of animal models of cancer and blocked corneal as well as **choroidal** [G] angiogenesis in animal models of ocular disease[20-25]. Inhibition of αvβ3 function in quail embryos disrupted vasculogenesis by blocking lumen formation and disturbing vascular patterning[26].

Integrin αvβ3 antagonists induce endothelial cell apoptosis, increase the activity of the tumour suppressor p53, increase levels of the cell cycle inhibitor p21WAF1/CIP1 and decrease levels of the anti-apoptotic protein bax[27]. Further studies show that αvβ3 antagonists activate a caspase 8-dependent cell death program[28]. Thus, a sizeable body of evidence indicates that integrin αvβ3 promotes angiogenesis and endothelial cell survival and that antagonism of this integrin suppresses angiogenesis by inducing endothelial cell apoptosis *in vitro* and *in vivo*. Ligation of endothelial αvβ3 integrin has also been shown to activate MAP kinase, focal adhesion kinase (FAK) and Src, among other kinases, resulting in cell proliferation, differentiation and migration[29].

The related integrin, αvβ5, promotes an angiogenic pathway that is distinct from that regulated by αvβ3. Anti-αvβ3 antibodies blocked angiogenesis induced by bFGF while antibodies that target αvβ5 blocked angiogenesis induced by VEGF in both the rabbit corneal eye pocket and the chick chorioallantoic membrane (CAM) assay[23]. The VEGF/αvβ5 pathway, but not the bFGF/αvβ3 pathway, is dependent on Src kinase and protein kinase C[30]. *In vivo* angiogenesis assays showed that bFGF and TNF-α depend on αvβ3 to initiate angiogenesis while αvβ5 is required for TGF-α and VEGF mediated angiogenesis. These data indicate unique roles for αvβ3 and αvβ5 integrins in angiogenesis and suggest that both could be important therapeutic targets. Some pathological conditions may depend on αvβ5 while other may depend on αvβ3. For example, integrin αvβ5 is more prevalent on cerebral cavernous malformations than is integrin αvβ3[31]. Additionally, only VEGF, which acts through αvβ5, rather than bFGF, which acts through αvβ3, promotes survival of developing retinal vessels[32] as well as vascular permeability[33-34]. As vascular permeability promotes tumor metastasis[33-34], these results

suggest that integrins that promote vascular permeability, such as αvβ5, may also promote tumor metastasis.

In contrast to the roles that are indicated by expression and function analysis, studies of integrin β3 null mice suggest that this integrin is absolutely required for vascular development[35]. While half of integrin β3 null mice survive embryogenesis, the other half die in utero, exhibiting intrauterine bleeding or defective placental development[35]. These mice exhibit apparently normal vessels in the brain and gut, as well as normal postnatal retinal neovascularization. However, male mice lacking β3 integrin exhibit coronary capillaries of irregular endothelial thickness, with endothelial protrusions into the lumen, and expanded cytoplasmic vacuoles[36]. These defective coronary vessels can be normalized by administration of inhibitors of VEGF or Flk-1, suggesting that enhanced VEGF signaling may compensate for the loss of β3 integrin[36]. In fact, β3 null mice exhibit enhanced tumor angiogenesis compared with normal mice[37], with strongly upregulated VEGFR2 expression and signaling.[38] Taken together, these studies suggest that compensatory VEGFR2 signaling changes may play a role in the survival of β3 deficient animals. In contrast to β3 null mice, integrin αvβ5 null mice exhibit completely normal development and normal angiogenesis[39], indicating that this integrin is not required for vascular development.

Genetic ablation of the alpha v subunit (which eliminates expression of integrins αvβ1, αvβ3, αvβ5, αvβ6 and αvβ8) suggest that in some mice, αv integrins are not required for blood vessel development in most tissues. Twenty percent of embryos lacking αv integrins survive to birth with normal blood vessels in many tissues. However, eighty percent of mice die *in utero* between E10.5 and E11.5 with defective placental blood vessels.[40] The remaining twenty percent of αv null mice die shortly after birth with severe brain and intestinal hemorrhage, with distended and leaky vessels in these tissues.[40] Thus, αv integrins appear to play key roles in embryonic development of blood vessels in tissues such as placenta and brain. Surprisingly, brain blood vessels developed normally in mice with Tie2-Cre-mediated endothelial cell specific deletion of αv integrins, but not in mice with Nestin-Cre and GFAP-Cre mediated embryonic central nervous system deletion of αv integrins[41]. Mice null for integrin β8 also exhibited brain blood vessel defects similar to αv null mice.[18] Together, these studies indicate that glial cell αvβ8 integrin is required for proper brain blood vessel development. The brain blood vessels of mice lacking integrin αv or β8 subunits exhibit enlarged, disrupted blood vessels, with defective apposition of glial and endothelial cells[18,42]. Interestingly, these studies suggest that glial cell integrins may play roles in establishing or maintaining the blood-brain barrier.

Results from studies of integrin antagonists indicate that αv integrins promote angiogenesis, while genetic deletion studies indicate that αv integrins are not required for angiogenesis. One hypothesis to explain this conflict is that αv integrins act as negative regulators of angiogenesis; once deleted in development, angiogenesis occurs at an accelerated rate. An alternative hypothesis is that animals lacking αv integrins develop compensatory changes in VEGF signaling that permit angiogenesis to occur during embryogenesis. New studies bring clarification to the much-disputed role of this integrin in angiogenesis. Importantly, knockin mutant animals expressing the point mutations Y747F and Y759F in the integrin β3 cytoplasmic tail developed normally, but exhibit reduced growth factor and tumor induced angiogenesis *in vivo*.[43] Mutant endothelial cells exhibit impaired adhesion, spreading, migration and tube formation, as well as impaired complex formation between VEGF receptor-2 and β3 integrin[43]. These results provide genetic evidence that integrin β3 plays an important role in promoting angiogenesis[43]. Together, these diverse results can be interpreted to indicate that integrin αvβ3 plays an important role in angiogenesis and that loss of expression of this integrin in development can be partially compensated for by upregulation of other angiogenesis signaling pathways.

How does integrin αvβ3 modulate angiogenesis? Unligated or antagonized αvβ3 integrin can inhibit cell survival. In a three-dimensional collagen matrix, αvβ3-bearing cells undergo apoptosis while cells deficient in αvβ3 survive longer.[44] As intact collagen is not a ligand for αvβ3, these studies indicated that unligated integrin αvβ3 provides a stimulus for apoptosis. This unligated integrin mediated cell death is caspase-8 dependent and is sometime called 'integrin-mediated death.'[28] Functioning as a biosensor, αvβ3 may promote angiogenesis in the ligated state, but when ligands are absent or ligand-binding is antagonized, αvβ3 may activate a death pathway to inhibit angiogenesis.

Thus, depending on the microenvironment and the ligation state, integrins promote or inhibit angiogenesis by positively or negatively regulating cell survival. Integrin αvβ3 appears to function as a regulator of angiogenesis by balancing opposing signals in the tumor microenvironment.

Fibronectin-binding Integrins

Fibronectin is key extracellular matrix protein that is deposited by endothelial cell during normal[45] and tumor angiogenesis[46]. Short fibronectin peptide loops containing the sequence Arginine-Glycine-Aspartic acid (RGD) interact with integrins such as α5β1, αvβ5 and αvβ3[11]. Fibronectin secreted by endothelial cells also contains the alternatively spliced EIIIA, EIIIB and IIICS domains, which bind to integrins α4β1 and α9β1.[13, 47] Fibronectin is essential for developmental angiogenesis as deletion of all fibronectin isoforms is early embryonic lethal, with yolk sac and other mesodermal tissue defects[48]. Deletion of only EIIIA and EIIIB alternatively spliced variants is also embryonic lethal with sever vascular defects that suppress placentation, yolk sac vessel formation and heart formation[49]. Thus fibronectin plays a key role in angiogenesis, as do many of its receptors, the β1 integrins.

Genetic ablation studies have demonstrated the critical role of β1 integrins in angiogenesis and vascular development. β1-integrin-null embryos die by E9.5-10.5 in utero due to implantation defects[50-51]. However, animals with an endothelial cell specific deletion of β1 integrin (Tie2Creβ1 floxed mice) die by E10.5 with severe vascular defects.[52] Integrin β1-null **teratomas [G]** grown in wild type hosts develop small host-derived vessels but no β1-null vessels[53]. Furthermore, endothelial cell proliferation and vessel branching is absent in β1-null embryoid bodies[53]. Thus, the β1 integrin family clearly plays a key role in angiogenesis.

Integrin α5β1 is poorly expressed on quiescent endothelium but its expression is significantly upregulated on endothelium during tumor angiogenesis in both mice and humans.[46] Expression of this integrin is also upregulated during corneal angiogenesis.[54] Integrin α5β1 expression is induced in response to a variety of angiogenic stimuli, such as bFGF, IL-8, and the ECM protein, Del-1, but not by VEGF[46,55]. Current evidence suggests that the activity of this integrin in angiogenesis is regulated by transcription[55]. Indeed, expression of α5β1 in endothelial cells (ECs) is regulated by a homeobox family transcription factor, HoxD3 which itself is induced in response to bFGF and other stimuli but not by VEGF[55]. Hox D3 antisense suppresses integrin α5β1 expression *in vitro* and *in vivo* while overexpression of Hox D3 upregulates α5β1 expression[55].

Antagonists of α5β1 inhibited tumor[46], corneal[54] and choroidal[56] angiogenesis in chicks and mice, thus leading to inhibition of tumor growth or tumor regression. Integrin α5β1 mediated adhesion promotes endothelial cell migration and survival *in vivo* and in vitro by suppressing the activity of Protein Kinase A (PKA)[57-58]. Activation of PKA by expression of the catalytic subunit of PKA or by exposure of cells to cAMP or **forskolin [G]** directly inhibits cell migration and stimulates apoptosis *in vitro* and *in vivo*. Thus, integrin α5β1 promotes endothelial cell survival during angiogenesis.

Embryonic deletion of the integrin α5 subunit induces early mesenchymal abnormalities, leading to lethality of α5-null embryos[59]. Embryos lacking integrin α5 have a truncated posterior and lack **posterior somites [G]**. These embryos also present with abnormal organization of the emerging extra embryonic and embryonic vasculature, and reduced complexity of the emerging vasculature[59]. Further studies using α5 null ES cells to grow teratocarcinomas showed decreased proliferation, increased apoptosis and decreased vascularization in teratocarcinomas derived from α5-null ES cells compared with controls[60]. *In vitro* growth of embryoid bodies lacking α5 integrins showed a delay and reduction in the formation of the early vascular plexus and formation of complex vascular structures[61]. Together, these data indicate a key role for α5β1 in vasculogenesis and angiogenesis.

Integrin α4β1, another important fibronectin receptor, is best known as a lymphocyte integrin involved in adhesion and extravasation of lymphocytes by binding to VCAM-1 expressed on inflamed endothelial cells. This integrin can bind both IIICS fibronectin and VCAM-1, a member of the immunoglobulin superfamily. Loss of integrin α4 during development leads to defects in placentation, heart development and coronary artery development, causing lethality between E10.0-E12.0[62]. However, due to the severe heart defects, it is not clear whether α4β1 plays a role in developmental angiogenesis.

Nevertheless, recent studies showed that integrin α4β1 is expressed on neovessels in murine and human tumors and in response to VEGF, bFGF, IL-1β and TNF-α[63]. Antagonists of this integrin blocked tumor neovascularization and decreased tumor growth in chick and murine models of tumor growth[63]. This integrin promotes adhesion of endothelium with VCAM-1 expressing vascular smooth muscle cells during blood vessel formation and antagonists of α4β1 induced cell death of both endothelial cells and **pericytes** [G][63]. Transient, but direct, contact between endothelial cells and pericytes during angiogenesis appears to play an important role in cell survival signaling in each cell type, although the exact signaling mechanisms remain unknown. As endothelial cells express the pericyte chemoattractant PDGF and pericytes express VEGF, it is possible that close proximity of the two cell types facilitated by integrin α4β1-VCAM interactions allows each cell type to respond to growth and survival signals emanating from the other cell type[63]. Thus, integrin α4β1-VCAM-1 dependent cell-cell attachment promotes the survival of both endothelial cells and pericytes during angiogenesis.

The most recent fibronectin-binding integrin to be found to play a role in angiogenesis is integrin α9β1[64-67]. This interesting integrin is structurally similar to integrin α4β1 but unlike α4β1, it is a receptor for a number of ECM proteins and cell surface receptors including tenascin-C, thrombospondin, osteopontin, IIICS fibronectin, VCAM and other ligands[64-67]. Integrin α9β1 is expressed on epithelia, osteoclasts, smooth muscle cells and also endothelial cells. Recent studies show that this integrin promotes VEGF-A stimulated angiogenesis by a unique mechanism; it directly binds VEGF-A 121 and blocking antibodies to α9β1 suppress VEGF-A induced angiogenesis[64]. However, other studies found that α9β1 bind directly to the N-terminus of thrombospondin and blocking antibodies to α9β1 inhibited angiogenesis induced by this thrombospondin fragment[65]. Integrin α9β1 null mice die 8-12 days after birth due to lethal defects in development of the lymphatic system; however, they do not exhibit obvious defects in development of blood vessels[68].

Fibronectin and several of its receptors clearly play central roles in promoting angiogenesis during development and during tumor growth. However, additional integrin subfamilies, such as the laminin family also regulate angiogenesis as described below.

Laminin-binding integrins

Similar to integrins αvβ3/αvβ5, results of studies of the expression and function of the laminin and collagen binding integrins α1β1 and α2β1 differ from in ovo genetic deletions. In normal animals, VEGF-A treatment upregulates expression of both α1β1 and α2β1 on vascular endothelial cells[69]. Function-blocking antibodies directed against both integrins reduced VEGF-1 induced angiogenesis *in vivo* and reduced tumor growth and angiogenesis[69]. However, integrin α1-null mice and α2-null mice are viable and fertile, indicating that these integrins are not required for angiogenesis in developing embryos. Tumors grown in α1β1 deficient mice grew more slowly and exhibited less angiogenesis, indicating an important role for this integrin in angiogenesis[70]. However, mice lacking α2β1 integrins exhibit enhanced B16F10 melanoma tumor growth and angiogenesis when compared to wildtype mice[71]. In contrast, LLC tumors did not exhibit increased angiogenesis or growth in alpha 2 null mice. Enhanced angiogenesis was attributed to an increase in VEGFR1 expression and function on α2 null endothelial cells. While B16F10 melanoma cells expressed high levels of the VEGFR1 binding growth factor PLGF, LLC cells expressed low levels of PLGF. Upon transfection with PLGF, LLC tumors grew more rapidly in α2β1 null animals compared with wildtype, suggesting that compensatory changes in growth factor expression and signaling can occur in animals with null mutations[71]. Integrins α1β1 and α2β1 appear to play important roles in tumor angiogenesis, although complete understanding of their roles is still developing.

The α6 integrin subunit can form heterodimers with either the β1 or the β4 subunit. Integrin α6β1 is expressed at high levels in capillary endothelial cells *in vivo*[72]. Antibody inhibitors of α6 integrin prevented endothelial cell tube formation *in vitro*, suggesting a role for α6 in the angiogenic process[72]. Endothelial cell migration and tube formation was also blocked by down-regulation of α6 expression in brain microvascular endothelial cells with siRNA[72]. Histological analysis reveals that the β4 subunit is expressed on human and murine tumor endothelium[73]. In addition, the α6β4 ligand, laminin 5, is also expressed by tumor blood vessels[73]. However, mice that are null for the β4 or the α6 subunit do not exhibit overt vascular defects but die immediately after birth in part due to severe skin blistering caused by passage through the birth canal[74-76].

In stably adherent cells, the β4 integrin mediates **hemidesmosome [G]** assembly[76]. However, in growth factor stimulated cells, hemidesmosomes do not form and the β4 subunit cytoplasmic tail is tyrosine phosphorylated in response to receptor tyrosine kinase activation of the Src family kinases. Upon phosphorylation of Tyr(1440) and Tyr(1422), the β4 subunit interacts with the SH2 domain of Shc, promoting Raf-ERK and Rac-JNK signaling and immediately early gene expression[77]. Mice with a targeted deletion of the C-terminal portion of the β4 subunit cytoplasmic tail (Δ1355) developed normally, but adult mice exhibited a reduced angiogenic response to bFGF and VEGF[73]. Spontaneous tumor growth in these animals was also suppressed, as was tumor angiogenesis. Integrin α6β4 did not affect proliferation of endothelial cells but was required for endothelial cell adhesion and migration. In addition, endothelial cell specific loss of β4 integrin suppressed nuclear translocation of phosphoErk and NFκB[73]. These studies suggest that integrin α6β4 is a novel target for anti-angiogenic therapies.

Integrin α6β1 is also expressed on endothelium; its expression can be confirmed by immunoprecipitation[76]. Unlike α6β4, which shows a preference for laminin-5, integrin α6β1 binds most laminin isoforms as well as other extracellular matrix proteins including Cyr61, TSP-1 and TSP-2[78]. As α6 integrin antagonists and α6 siRNA constructs inhibit angiogenesis, it is possible that integrin α6β1 promotes angiogenesis. However, as these agents can block the function of both integrins α6β1 and α6β4, it is not yet clear what role integrin α6β1 plays in tumor angiogenesis.

The studies described here show that endothelial cell integrins that bind to diverse extracellular matrix ligands promote angiogenesis during embryonic development and tumor growth. Embryonic deletion of many of these integrins does not lead to an embryonic lethal phenotype, indicating that many integrins have overlapping functions during development. However, as antagonists or deletion of these integrins suppresses tumor angiogenesis, each integrin can contribute to angiogenesis in at least some tumor microenvironments.

Integrins on bone marrow derived cells

Integrins on bone marrow-derived immune cells can also promote angiogenesis by facilitating myeloid cell and endothelial progenitor cell homing to tumors (Figure 2). Circulating bone marrow derived cells migrate into tumors in response to secreted chemokines and cytokines. Monocytes can then differentiate into pro-angiogenic secreting macrophages. Myeloid cells express a number of functional integrins (α2β1, α4β1, α5β1, αvβ3, αvβ5, αMβ2 (CD11b) and αXβ2 (CD11c). A subset of these integrins regulate extravasation of these cells from the blood stream and their migration within neovascular microenvironments.

Recent studies indicate that integrin α4β1, a receptor for VCAM and CS-1 fibronectin, selectively promotes the homing of both endothelial progenitor cells and monocytes to neovascular tissue[79-80]. Human CD34+ and murine Lin-Sca1+ progenitor cells as well as human and murine bone marrow-derived myeloid cells (CD14+ CD11b+) adhered to endothelial cells *in vitro* and to tumor endothelium *in vivo* via integrin α4β1[79-80]. Treatment of mice bearing Lewis lung carcinoma tumors with antagonists of integrin α4β1 significantly suppressed the number of monocytes and progenitor cells found within tumors and reduced blood vessel density[86]. These studies suggest that the suppression of monocyte and progenitor cell homing to tumors by integrin α4β1 antagonists could be a useful supplementary approach to suppress tumor angiogenesis and growth.

Other integrins can also mediate homing of precursor cells to sites of neovascularization. The adhesion of circulating endothelial precursor cells to endothelial monolayers was shown to be integrin β2 dependent. Hematopoietic progenitor cells (Sca+ Lin-cells) from β2-integrin deficient mice exhibited reduced homing to sites of ischemia[81-82]. Together, these studies identified key functions for integrins during precursor cell/monocyte homing to angiogenic sites.

The platelet integrins αIIbβ3 and α2β1 may indirectly regulate angiogenesis by promoting platelet deposition within tumors and damaged tissues. Platelets release several pro-angiogenic factors, which are stored in **alpha granules** [G], such as VEGF-A, SDF-1, and sphingosine-1-phosphate, suggesting that integrins on platelets may also play roles in angiogenesis[83-84]. Thus, integrins on bone marrow derived cells can play key roles in angiogenesis.

Integrins in Lymphangiogenesis

Lymphatic vessels form a network that drains fluid and cells from tissues; these vessels are required for tissue homeostasis. Lymphatic capillaries are lined by loosely associated endothelial cells without a covering of pericytes or vascular smooth muscle. This structure allows ready passage of immune cells and tumor cells into the lymphatic system. Indeed, lymph nodes are typically the first organ to exhibit tumor metastasis, and sentinel node monitoring is used extensively to detect metastases.

Analysis of the role of lymphatics in tumor growth and metastasis had been hindered until recently by the absence of lymphatic markers. Recent identification of specific lymphatic markers, such as the transcription factor Prox-1 and the CD44 homolog lymphatic vessel hyaluronan receptor-1 (LYVE-1)[85-86], has made it possible to study mechanisms regulating

lymphangiogenesis. The growth factors VEGF-C and VEGF-D, which can be expressed by tumor cells or macrophages in tumors, promote growth of the lymphatic vessel network by activating the lymphatic endothelial cell receptor VEGFR-3[87].

To date, evidence is mounting regarding roles for integrins in lymphangiogenesis (Figure 1). Integrin α9β1 is required for development of the fully functional lymphatic system because mice deficient in α9β1 integrin die 6 to 12 days after birth due to chylothorax, an accumulation of lymph in the pleural cavity[68]. Integrin α9β1 plays a role in growth factor mediated lymphangiogenesis as Prox-1, a lymphatic endothelial cell selective transcription factor coordinately upregulates integrin α9β1 and VEGFR3 expression and endothelial cell motility *in vivo*[88]. Recent studies show that this integrin promotes VEGF-C and D stimulated cell migration by directly binding these growth factors[89]. Importantly, antagonism of α9β1 suppresses VEGF-C induced motility. Taken together, these studies indicate that α9β1 plays unique yet critical roles in lymphangiogenesis.

Other studies have shown that integrins α1β1 and α2β1 are expressed on lymphatic endothelium in healing wounds in response to VEGF-A. Inhibition of these integrins blocked lymphangiogenesis in these wounds[90]. Integrin α5β1 is expressed by a subpopulation of lymphatic vessels in the inflamed cornea and small molecule antagonists of this integrin inhibited inflammatory lymphangiogenesis[91]. Integrin α4β1 is highly expressed on tumor lymphatic endothelium[92] and antagonists of this integrin can block lymphangiogenesis and tumor metastasis. In contrast, αv integrins appear to play little or no role in lymphangiogenesis and integrin α5β1 appears to play no role in tumor lymphangiogenesis[92]. Thus, several integrins appear to play important roles in lymphangiogenesis yet the profile of integrins regulating lymphangiogenesis distinct from those regulating angiogenesis. Importantly, antagonists of these integrins may be useful in preventing tumor metastasis by blocking lymphangiogenesis.

Potential clinical applications

Preclinical studies suggested that antagonists of several integrins might be useful to suppress tumor angiogenesis and growth either alone or in combination with current cancer therapeutics. While antagonists of several integrins are undergoing preclinical evaluation and development, only a handful of integrin-based drugs have so far been tested in the clinic. Integrin antagonists include function-blocking antibodies, peptides and organic small molecules. Function-blocking anti-integrin monoclonal antibodies typically have high affinity and specificity and have been well characterized for years. These were the first integrin antagonists to reach the clinic. Cyclic peptide antagonists have also been evaluated in clinical trials. Small organic inhibitors, particularly orally active drugs, are the most cost effective therapeutics but the development of selective and high affinity integrin inhibitors is time consuming. Hence, no organic small molecules antagonists of integrins have yet entered clinical trials.

Of the several integrin antagonists undergoing clinical evaluation for cancer treatment, all have proven nontoxic, including Abegrin (Medi-522), a **humanized [G]** anti-αvβ3 antibody, CNTO95 a human αvβ3/αvβ5 antibody, Volociximab, a chimeric mouse/human anti-α5β1 antibody, Cilengitide, a cyclic peptide inhibitor of integrins αvβ3/αvβ5 and ATN161, a non-RGD based peptide inhibitor of α5β1 (Table 2). These agents are likely nontoxic because the targeted integrins are only expressed or activated in remodeling tissues such as tumors.

Abegrin, or MEDI-522, was the first anti-integrin therapeutic to be tested in clinical trials for cancer. It is a humanized version of the anti-integrin αvβ3 monoclonal antibody LM609, which has been shown to block tumor angiogenesis by inducing apoptosis in newly formed endothelial cells. A Phase I study showed that the early version of this drug, Vitaxin, had very low toxicity and was well tolerated[93-94]. When tested on patients with metastatic cancer who had failed

other treatments, Vitaxin led to disease stabilization without toxicity. However, in a second clinical trial, use of Vitaxin on patients with **leiomyosarcoma [G]** did not suggest anti-tumor activity. In 2001, Medimmune began clinical trials using Vitaxin (renamed MEDI-522/ Abegrin) and in 2003 initiated Phase II trials in patients with advanced metastatic melanoma and in patients with metastatic prostate cancer[95]. In one melanoma trial, 112 patients with stage IV melanoma received 8 mg/kg MEDI-522 each week. Of these patients, 57 also received 1000 mg/m^2 **dacarbazine [G]** once every 3 weeks while the rest (n=55) did not. The overall survival was 12.7 months for MEDI-522 + dacarbazine and 9.4 months for MEDI-522 alone. Both groups showed prolonged survival when compared with a historical control[95]. A recent study in patients showed that MEDI-522 showed functional efficacy by reducing focal adhesion kinase activity in patients' blood vessels[96]. Based on these results, Phase III cancer clinical trials are in the planning stages.

On the basis of preclinical studies showing both integrins αvβ3 and αvβ5 regulate angiogenesis, a human monoclonal antibody directed against both αvβ3 and αvβ5 integrins, CNTO 95, was developed by Centocor. CNTO 95 reduced angiogenesis and tumor growth in human melanoma xenografts in nude mice and rats[97]. Preclinical safety studies on cynomolgus macaques (*Macaca fascicularis)* showed no toxicity[98]. This antibody has completed phase I safety trials with a favorable safety profile, extended treatment offered to one third of patients and one patient exhibiting a partial response[99]. CNTO95 is now under evaluation in Phase I/ II clinical trial for the treatment of melanoma (http://clinicaltrials.gov/show/NCT00246012). As CNTO95 inhibits both integrins αvβ3 and αvβ5, two of the integrins that promote tumor angiogenesis, it may have wide-spread clinical utility. Additionally, most carcinoma cells express integrin αvβ5, which has been shown to promote tumor cell invasion[100]. Targeting the alpha v integrins may thus block both tumor cell invasion and metastasis and tumor angiogenesis.

For these reasons, a cyclic RGD-peptide antagonist of αvβ3/αvβ5, Cilengitide (EMD 121974) has been developed as a cancer therapeutic. Phase I clinical trials showed favorable safety profiles and no dose-limiting toxicities[101-102]. In addition, Cilengitide was shown to enhance radiotherapy in cancer patients[103]. This drug was evaluated in Phase I/IIa clinical trials for glioblastoma and significantly enhanced progression free survival was observed[101]. On this basis, as of 2007, E. Merck was planning to evaluate Cilengitide in Phase III trials for glioblastoma. Cilengitide is currently in phase II trials for glioblastoma, non-small cell lung cancer, melanoma and pancreatic cancer[104-106]. Therefore, three distinct alpha v integrin targeting drugs offer promise as cancer therapeutics.

Since beta 1 integrins also play significant roles in angiogenesis, targeting these integrins in addition to alpha v integrins may be provide useful benefit in suppressing angiogenesis and tumor growth. Antagonists of one integrin, the alpha 5 beta 1 integrin have undergone clinical testing. A chimeric mouse/human anti-α5β1 antibody, M200 (volociximab), developed by Protein Design Lab and now partnered with Biogen-Idec Pharmaceuticals, has shown low toxicity in Phase I studies. M200 was evaluated in Phase II trials for metastatic melanoma, renal cell carcinoma and non-small cell lung cancer[107-108]. In renal cell carcinoma studies M200 was well-tolerated at a dose of 10 mg/kg every 2 weeks and stable disease was noted in 87% of patients. In a melanoma trial in combination with **DTIC** [G], the antibody was well-tolerated and anti-tumor activity was noted in 62% of patients

Another drug in clinical trials, ATN-161, is a peptide inhibitor of integrin α5β1. In animal models of colon cancer, ATN-161 reduced metastases and improved survival when combined with chemotherapy[109]. In Phase I safety trials, ATN161 was well tolerated and several patients exhibited stable disease. ATN-161 is currently in Phase II clinical trials for multiple myeloma

and other tumors[110]. Thus, these two integrin α5β1 inhibiting drugs may offer future benefit to cancer patients.

However, as many integrins can promote angiogenesis, it is not yet clear whether targeting one or more than one will have the most significant impact on tumor angiogenesis and growth. It is likely also that integrin antagonists may be combined with other angiogenesis inhibitors such as VEGF inhibitors like Avastin.

Integrin Targeted Tumor Imaging and Treatment

Integrin ligands are also under investigation as tumor endothelium targeted diagnostic agents. Both peptide and antibody based diagnostic agents targeting RGD binding integrins or αv integrins have been evaluated in animal models of cancer. **PEGylated [G]** cyclic RGD peptides labeled with ^{18}F or ^{64}Cu were effective in imaging xenograft brain and breast tumors with a low signal to noise ratio. These imaging approaches have been able to identify xenograft tumors as small as 1.5mm in diameter[111-112]. Paramagnetic polymerized liposomes conjugated to an anti-αvβ3 antibody were also used to detect neovascular tumors in experimental rabbit tumors[113]. Integrin αv-targeted ultrasound **microbubbles [G]** also preferentially bound neovasculature at the periphery of experimental tumors[114]. Thus integrin targeting is proving useful in tumor imaging. It is possible that agents targeting distinct integrins may be useful in pre-classifying patients for receipt of anti-integrin drugs.

Integrin targeting agents may also be useful in selectively delivering chemotherapeutics or gene therapeutic agents to tumors, thus minimizing global toxicities. For example, an integrin αvβ3 and αvβ5 targeted liposome (nanoparticles) was used to deliver a mutant Raf construct to tumors in animals; this Raf construct induced endothelial cell apoptosis and subsequent tumor suppression[115]. Integrins have thus also proven to be effective targets for delivery of imaging and therapeutic agents.

Conclusions/Perspectives

Blood and lymphatic vessels play critical roles in promoting tumor growth and metastasis. A substantial body of experimental evidence indicates that integrins regulate endothelial cell migration and survival during angiogenesis and lymphangiogenesis. In addition, integrins promote monocyte trafficking to tumors and subsequent angiogenesis and lymphangiogenesis. As angiogenesis promotes tumor growth and metastasis and lymphangiogenesis promotes tumor metastasis by providing conduits for tumor escape through the lymphatic system, antagonists of these integrins may be useful in blocking tumor metastasis in patients.

Although there is much experimental evidence supporting roles for integrins in tumor angiogenesis and lymphangiogenesis, conflicts between integrin knockout and in vivo tumor studies need to be resolved in the future. Why do many integrin deletion mutants develop normally yet exhibit altered tumor angiogenesis? Future comparisons of embryonic and tumor angiogenesis will address whether compensatory molecular changes occur in surviving mutant animals or whether there are quantitative but non-lethal changes in blood vessel densities in mutant animals.

As several integrin-targeted therapeutic agents are in clinical trials for cancer therapy, future clinical studies will likely determine whether integrin inhibitors will be best used against select tumors, such as those in which tumor cells themselves express the targeted integrin. In addition, integrin targeted nanoparticles will continue to be developed for the imaging of tumor vasculature and delivery of gene therapy or chemotherapeutic drugs. As integrins are clearly a family of critical and fundamental regulators of angiogenesis and lymphangiogenesis, the future of integrin antagonists in cancer therapy is promising.

Text Box 1: Regulation of Integrin Activation

Integrin activity during angiogenesis can be modulated by integrin expression or by growth factor or chemokine receptor signaling, which alters integrin conformation ("inside-out" signaling). Crystallography, nuclear magnetic resonance and electron microscopy studies suggest that the globular region formed by the N-termini of the α and β chains is bent towards the membrane and the cytoplasmic regions of the two subunits are closely associated with one another in an inactive integrin[116-119]. Integrin activation is associated with an unbending and elongation of the dimer and separation of the cytoplasmic domains[116-119]. This allows interaction of integrin cytoplasmic domains with intracellular proteins and permits integrin-mediated signal transduction. Once activated, integrins bind ligands, cluster and initiate their own signaling cascades that lead to cell migration and survival. The activity of many endothelial cell integrins during angiogenesis is regulated primarily at the transcriptional level. The expression of integrins α1β1, α2β1, α4β1, α5β1 and αvβ3 is strongly induced by pro-angiogenic growth factors or chemokines[20-23, 67, 73]. However, other integrins are activated by receptor-mediated signaling. Integrin αvβ5 is constitutively expressed on vascular endothelium, yet is only functionally activated by VEGFR2 mediated signal transduction[31]. In circulating cells such as monocytes and other leukocytes, integrins are generally inactive until cells are stimulated by chemokines, hormones or other factors. For example, integrin α4β1 activity on leukocytes can be stimulated by chemokine signaling[127]. Additionally, integrin αIIbβ3 is inactive on resting platelets but becomes activated from within when an external stimulus such as thrombin or epinephrine binds a cell surface receptor and induces the conformational change in the integrin cytoplasmic domains. It then binds its ligand fibrinogen, leading to platelet aggregation[128]. Thus, integrins roles in angiogenesis can be controlled either by expression or by intracellular signaling (inside-out signaling). Thus in tumors, the expression of VEGF and other pro-angiogenic factors can continuously stimulate integrin expression and activity and stimulate unregulated angiogenesis. A schematic representation of the structure of an inactive and active integrin is shown (adapted with permission from ref. [123]).

Textbox 2: Integrin regulation of cell migration and focal adhesions

Integrins "integrate" signals from the extracellular matrix to the intracellular cytoskeleton in focal adhesions. Integrins have short cytoplasmic tails and no intrinsic enzymatic or kinase activities. To integrate signals and activate intracellular signaling pathways, integrins co-cluster with serine, threonine and tyrosine kinases, phosphatases and adaptor proteins in focal adhesions. Focal adhesion complexes are comprised of integrins, protein kinases such as focal adhesion kinase (FAK), Src and many other kinases, adaptor proteins such as Shc, signaling intermediates such as PI-3-kinase, Rho and Rac GTPases and actin binding cytoskeletal proteins such as talin, α-actinin, paxillin, tensin and vinculin[15-16]. Integrin signaling promotes cell migration by providing traction along the extracellular matrix and by promoting actin remodeling through the Rho family small GTPases. This actin remodeling leads to cytoplasmic flow in the direction of cell migration and cell body retraction at the trailing end of the cells. Individual components of integrin-mediated signaling cascades, such as FAK, Shc and Raf, play essential roles in angiogenesis. For example, focal adhesion kinase (FAK) is a mediator of signal transduction by integrins and growth factor receptors in endothelial cells. Overexpression of FAK in mice promoted angiogenesis[122]. In contrast, conditional deletion of focal adhesion kinase in endothelial cells led to defective angiogenesis in the embryos, yolk sac, and placenta, impaired vasculature and associated hemorrhage, edema, and developmental delay, and late embryonic lethality[123]. In addition, in vitro deletion of FAK in ECs isolated from floxed FAK mice exhibited reduced tube formation, cell survival, proliferation, and migration in vitro[123]. These results indicate that FAK plays a critical role in angiogenesis and vascular

development. In addition, Shc, an important adaptor protein that potentiates MAP kinase pathway signaling is activated by both integrins and growth factor receptors and plays critical roles in early vascular development. Shc is primarily expressed during early mouse development in the developing heart and endothelium.[124] Shc null animals died between E10.5 and 11.5 with defects in heart and blood vessel development and isolated Shc null cells exhibited migration deficiencies.[124] Like Shc, Raf-1 is an integral component of the MAP kinase signaling pathway. This signaling intermediate is activated by integrins and is critical for vascular morphogenesis.[125] Mice null for Raf-1 die during early development by E11.5 with vascular defects in the yolk sac, placenta and head.[125] Thus, integrin mediated signaling likely plays important roles in vascular development adult angiogenesis. A schematic representation of integrin signaling and focal adhesion components is shown.

Textbox 3: The links between inflammation, wound-healing and cancer

Inflammation is increasingly thought to play important roles in tumor initiation, progression and metastasis[126]. Inflammation is caused by tissue injury, ischemia, toxins and other damaging stimuli. Injured tissues release chemokines and growth factors that attract leukocytes and upregulate adhesion molecules on the surface of endothelium in injured tissues to promote wound-healing. When the release of these factors is chronic, invasion of leukocytes into tissues is sustained and the further release of chemokines and factors can induce oxidative damage, DNA mutations, tumor development, tumor angiogenesis and lymphangiogenesis and subsequent metastasis. A key example is the increased risk of developing colon carcinoma in patients with chronic inflammation such as inflammatory bowel disease[126]. Substantial evidence supports the role of inflammation in tumor angiogenesis. The number of macrophages in tumor tissues is significantly greater than in normal tissues[127-128]. Importantly, high TAM densities indicate poor prognoses in breast, prostate, ovarian and cervical cancer[129-134]. Tumor associated macrophages promote neovascularization by releasing potent pro-angiogenic cytokines and growth factors, such as VEGF, tumor necrosis factor -α (TNF-α), interleukin-8 (IL-8) and bFGF[135-137]. Additionally, TAMs also express a broad array of proteases known to play roles in the angiogenic process. These proteases include urokinase-type plasminogen activator (uPA) and the matrix metalloproteinases MMP-2, MMP-7, MMP-9 and MMP12[138-141] that remodel and breaking down the extracellular matrix (ECM). Tumors produce factors such as monocyte chemoattractant protein-1 (MCP-1, or CCL2) and RANTES (CCL5) that increase the infiltration of TAMs in several primary tumors including breast, ovarian, melanoma, and glioblastoma[142-144]. Besides stimulating tumor angiogenesis and lymphangiogenesis, inflammatory chemokines, such as IL-6, or the genes that regulate them, such as IKKbeta and IKKalpha, also promote tumor progression to malignancy by directly stimulating tumor cell activation or cancer stem cell proliferation[146-150].

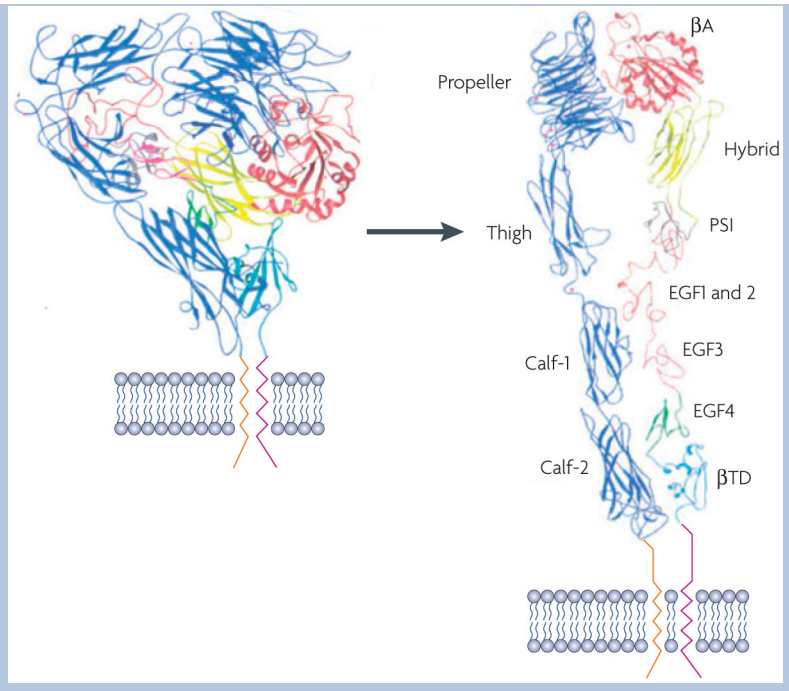

FIG. Box 1.

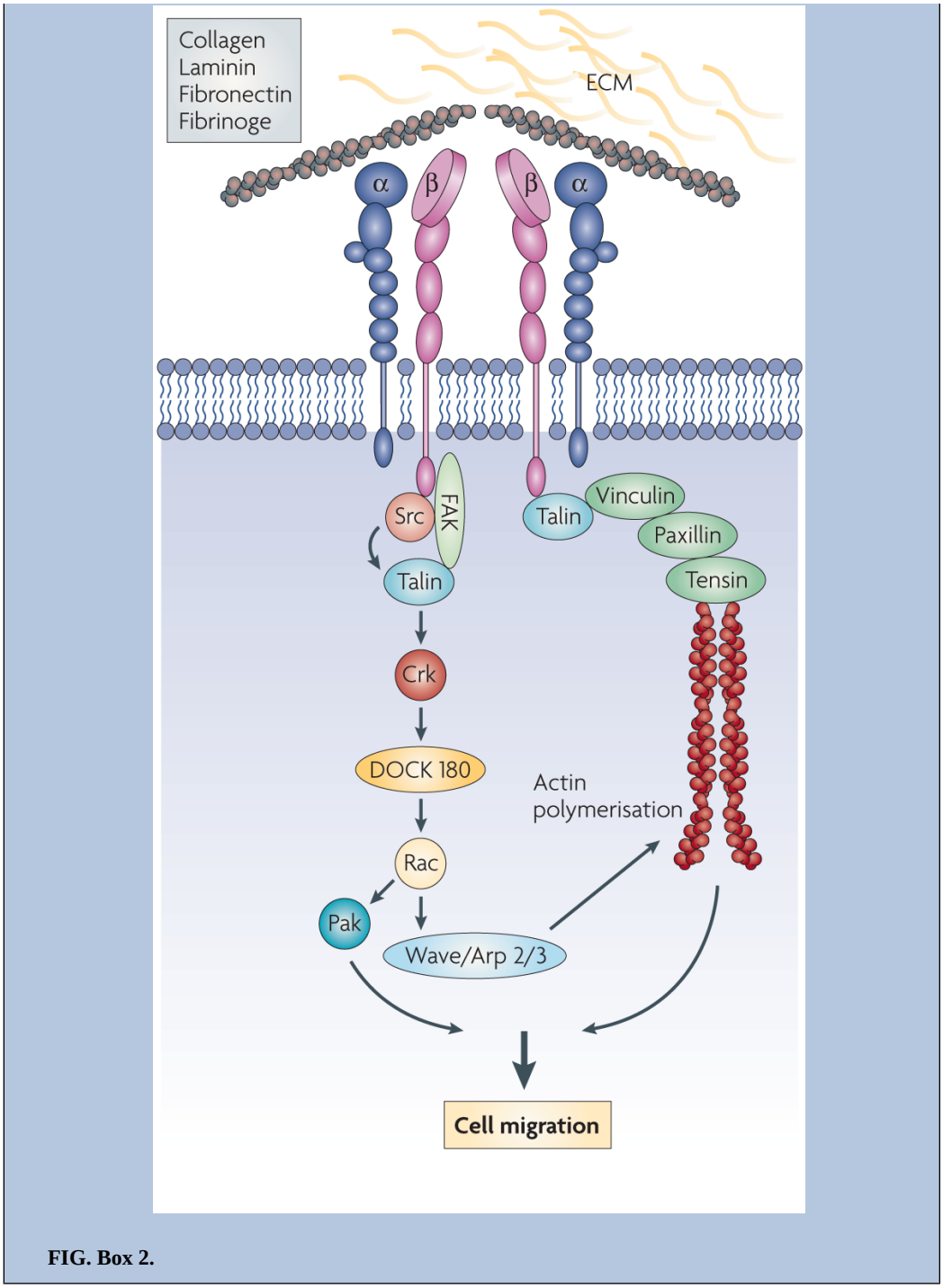

FIG. Box 2.

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GLOSSARY

Peyer's Patches, Secondary lymphoid organs named after the 17th century Swiss anatomist Joseph Conrad Peyer that are comprised of round lymphoid follicles in the mucosa of the small intestine

angiogenic switch, The induction of new blood vessel sprouting at an early time point in tumor development that leads to rapid, exponential tumor growth

chick chorioallantoic membrane, A thin, highly vascularized fetal membrane formed by fusion of the chorion and allantois in fertilized chicken eggs that is often used to evaluate pro- and anti-angiogenic agents

choroidal angiogenesis, The development of new blood vessels in the highly vascular area of the eye that lies between the retina and the sclera

teratomas, Germ cell tumors comprised of undifferentiated and differentiated cells derived from the three germ layers, mesoderm, ectoderm and endoderm; teratomas may include hair, teeth and other complex structures

forskolin, A diterpene derived from the Indian Coleus plant that raises cAMP levels in cells by activating adenyl cyclase

posterior somites, Cuboidal, segmented masses of mesoderm organized in pairs and distributed along the developing neural tube. Posterior somites give rise to the thoracic, lumbar, and sacral vertebrae

pericytes, A mesenchymal cell precursor to vascular smooth muscle that associates with endothelial cells during angiogenesis and provides support to small capillaries

hemidesmosome, An organized adhesive structure on the surface of epithelial cells comprised of integrin α6β4 attached on the exterior of the cell to laminin and on the interior of the cells to plectin and cytosolic keratins

alpha granules, Endosomes or granules in platelets that contain growth factors such as VEGF, TGFbeta and PDGF

humanized antibody, A synthetic monoclonal antibody comprised of a human antibody backbone fused with the antigen recognition regions of a mouse monoclonal antibody through recombinant DNA techniques, which is developed to eliminate immunogenic sequences

leiomyosarcoma, A neoplasm comprised of tumor cells arising from smooth muscle (sarcoma) and frequently found in the stomach and small intestines

Dacarbazine, A chemotherapeutic, DNA alkylating agent used in the treatment of malignant melanoma and Hodgkin's disease

DTIC, An abbreviation for Dacarbazine, a chemotherapeutic agent

PEGylated, Covalently modified with poly(ethylene glycol) to make a hydrophobic drug more soluble and to mask a drug from the host immune system

Microbubbles, Small (3 μm or less) gas-filled bubbled that serve as contrast enhancing agents in diagnostic medical ultrasound imaging

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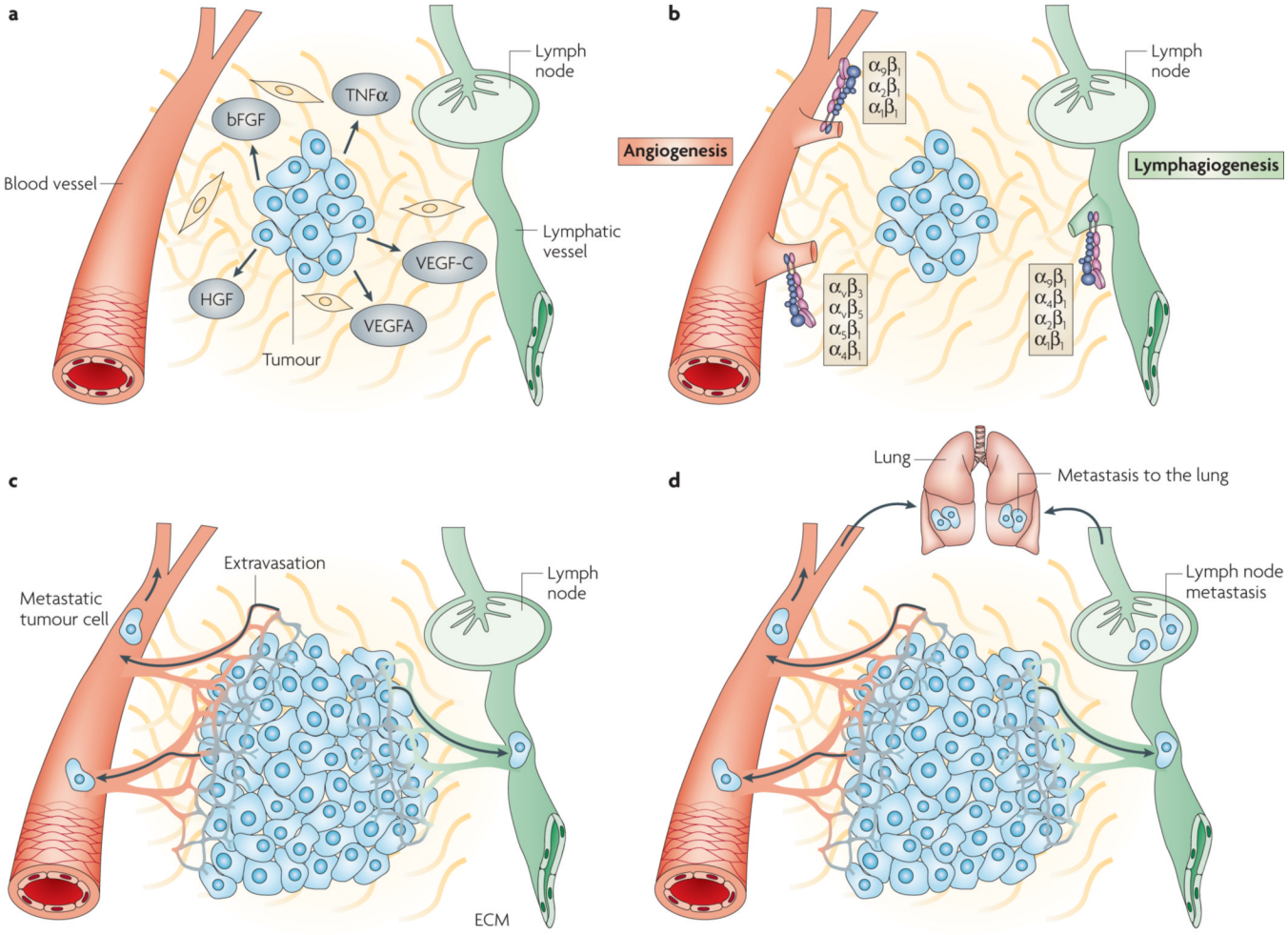

Figure 1. Mechanisms regulating angiogenesis and lymphangiogenesis

(A) Tumor cells near pre-existing blood vessels secrete growth factors and chemokines such as VEGF-A, bFGF, and TNFα that stimulate quiescent vascular endothelium to enter the cell cycle. Tumors also secrete factors such as VEGF-C, VEGF-A or HGF that stimulate the growth of new lymphatic vessels in the peritumoral space. (B) These growth factors activate or upregulate expression of integrins such as α1β1, α2β1, α4β1, α5β1 and αvβ3 on blood vessels and α4β1, α9β1, α2β1 and α1β1 on lymphatic vessels. Tumor derived VEGF-C also promotes new lymphatic vessel growth in draining lymph nodes. (C) These integrins then promote endothelial cell migration and survival during invasion of tumor tissue, resulting in the creation of new vessels sprouts. The new blood vessels promote tumor growth by removing waste products and providing nutrients. These new blood and lymphatic vessels also provide an avenue for tumor metastasis. (D) Lymphangiogenesis promotes metastasis to lymph nodes and, sometimes, more distant tissues such as lung, while angiogenesis promotes metastasis to local and distant sites, such as lung.

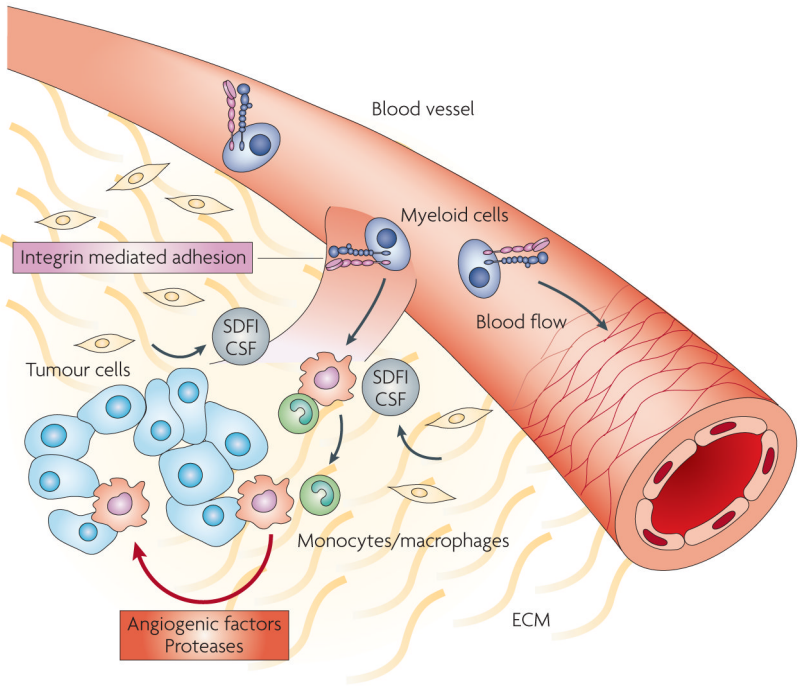

Figure 2. Myeloid cells promote angiogenesis

Myeloid precursor cells adhere to angiogenic endothelium via activated α4β1 or β2 integrins. A variety of tumor or stromal derived factors, including stromal derived factor 1 (SDF-1), colony stimulating factors (CSF), and others mobilize myeloid cells and activate integrins, promoting extravasation into the tumor environment. In the presence of other cytokines and growth factors, these cells differentiate into macrophages, which support tumor growth by expressing VEGF, other pro-angiogenic factors and proteases.