JCB Mini-Review

Tiny dancers: the integrin–growth factor nexus in angiogenic signaling

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A vital step in growth factor–driven angiogenesis is the coordinated engagement of endothelial integrins with the extracellular matrix. The molecular mechanisms that partner growth factors and integrins are being elucidated, revealing an intricate interaction of surface receptors and their signaling pathways.

Blood vessel formation is a dynamic process that involves interactions between soluble mediators, adhesive substrates, and endothelial cell surface receptors. Endothelial cell activation is a necessary first step in angiogenesis, which triggers the recruitment of smooth muscle cells and pericytes to newly formed vessels. Two growth factor families activate this initiating pathway in angiogenesis, the vascular endothelial growth factors (VEGFs)* and fibroblast growth factors (FGFs) (for review see Cross and Claesson-Welsh, 2001). VEGF-A, a factor that was initially identified based on its ability to increase vascular permeability and endothelial cell proliferation, is required for angiogenesis during development and is a necessary stimulus for hypoxia-induced angiogenesis. Four alternatively spliced isoforms of VEGF-A exist that bind two receptor tyrosine kinases, VEGF receptor (VEGFR)-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), on the surface of endothelial cells. The FGF family is even more fecund, consisting of at least 20 members that act on four separate receptors. Binding of VEGFs and FGFs to their respective receptors triggers receptor tyrosine phosphorylation followed by recruitment of intracellular adaptor proteins and activation of signaling molecules (Fig. 1). Through alterations in lipid metabolism, intracellular calcium levels, and protein kinase and phosphatase activities, growth factors elicit the pleiotrophic events necessary for new vessels to sprout from preexisting ones.

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*Abbreviations used in this paper: bFGF, basic FGF; ECM, extracellular matrix; FAK, focal adhesion kinase; MAPK, mitogen-activated protein kinase; PI, phosphatidylinositol; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

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Endothelial cell migration, proliferation, and the formation of new lumen during angiogenesis require coordinated interactions with the extracellular matrix (ECM). Growth factors regulate these interactions, in part, by stimulating the secretion of enzymes that degrade and alter ECM. Endothelial cell adhesion molecules, such as the integrins, are also required to coordinate interactions with the ECM. Integrins are heterodimeric cell surface adhesion receptors that mediate cell–cell and cell–ECM interactions and control cell migration, differentiation, proliferation, and survival by generating intracellular signals similar to those evoked by growth factors (for review see Aplin et al., 1998). Two lines of evidence suggest that endothelial αV integrins in particular play a key role in regulating angiogenesis. First, exogenously administered antibody and small molecule inhibitors of αV integrins decrease or prevent angiogenesis of tumors, retinal vessels, skin, and arthritic joints in a wide variety of animal and experimental models. Second, αV integrins appear to serve as cellular receptors for several endogenous pro- and anti-angiogenic proteins. In this mini-review, we examine recent insights derived from studies of the integrin–growth factor receptor nexus that help to understand how convergent and divergent signaling pathways mediate angiogenic events. We also consider studies which indicate that angiogenic integrins serve as a crucial switch to regulate endothelial cell survival and destruction.

Distinct angiogenic pathways as defined by specific growth factor–integrin pairs

The discovery by Cheresh and coworkers that antagonists specific for αVβ3 or αVβ5 selectively block angiogenesis induced by bFGF and VEGF, respectively, provided some of the first support for a role of αV integrins in angiogenesis and led them to postulate the existence of two separate angiogenic pathways (Friedlander et al., 1995). They observed that antibody antagonists of αVβ3 abolished basic FGF (bFGF)- and tumor necrosis factor α–stimulated angiogenesis but only partially affected the response to VEGF, whereas antagonists of αVβ5 inhibited VEGF-, TGFα-, and phorbol ester–induced angiogenesis. Cheresh and coworkers further distinguished the pathways based on pharmacologic susceptibility by demonstrating that inhibitors of PKC (Friedlander et al., 1995) and the tyrosine kinase Src

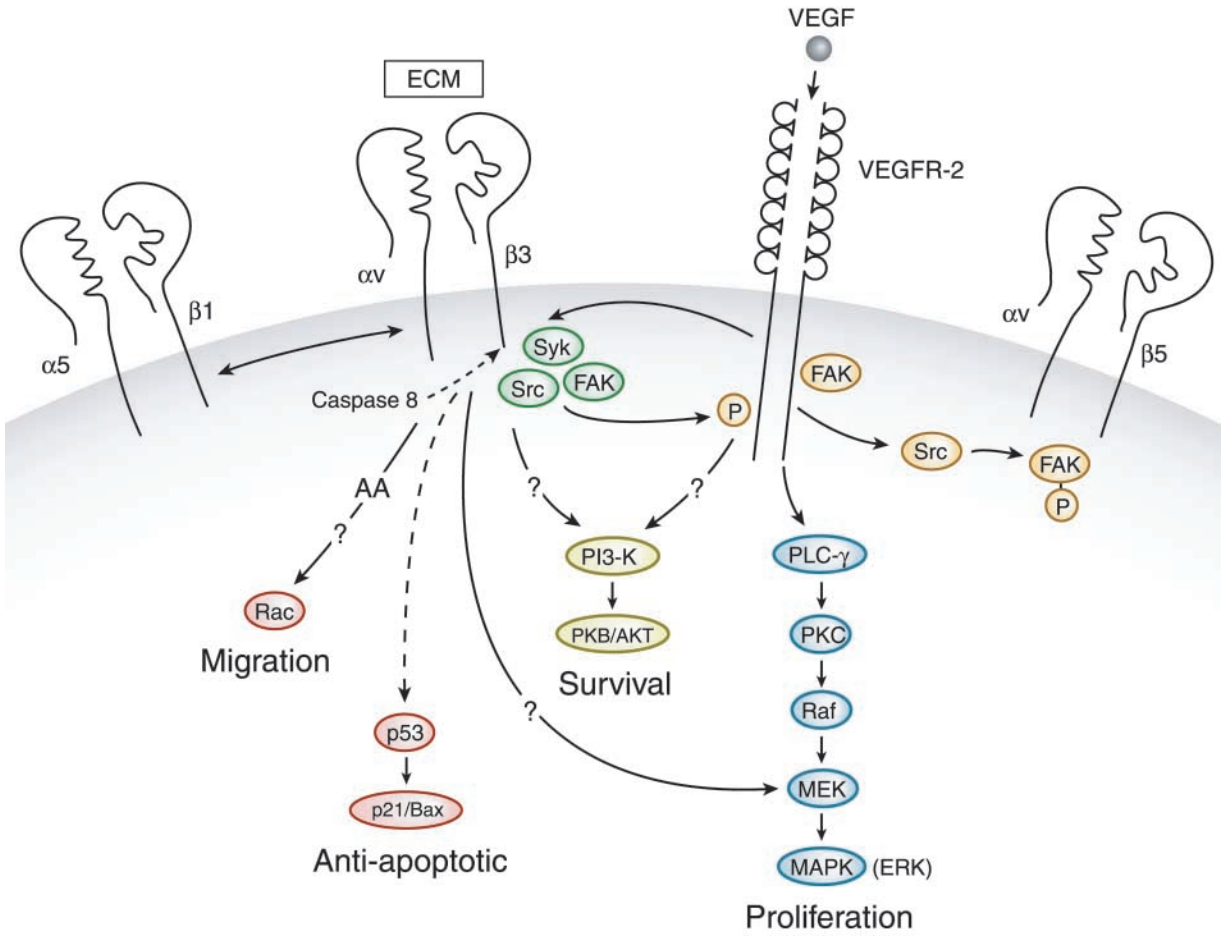

Figure 1. **Cross-talk between growth factors and integrins in endothelial cells.** VEGF binding to VEGFR-2 results in receptor dimerization and phosphorylation of specific tyrosine residues within the intracellular domain of the receptor. PLCγ, Sck, and VRAP (not depicted) all interact directly with VEGFR-2. The mechanism of activation of FAK, Src, MAPK, PI 3-kinase, and AKT by VEGFR-2 is less clearly understood. Recent work indicates that VEGF-mediated Src activation promotes FAK association with αVβ5. The extracellular domain of β3 directs association of αVβ3 with VEGFR-2. Engagement of either VEGFR-2 or αVβ3 enhances the function of the reciprocal receptor. Binding of ECM proteins to αVβ3 triggers phosphorylation of tyrosine residues located in the intracellular domain of the β3 chain and induces receptor clustering. Signaling molecules activated by ligation and/or clustering of αVβ3 include FAK, Src, MAPK, PI 3-kinase, and Rho family members. Recent evidence suggests a role for an unidentified arachidonic acid (AA) metabolite in αVβ3 activation of Rac. Phosphorylation of intracellular tyrosine residues of VEGFR-2 occurs in response to αVβ3 ligation. The p53/bax pathway linked to apoptosis is suppressed by αVβ3 engagement. The proapoptotic mediator caspase 8 may be activated by unligated αVβ3-dependent membrane recruitment. Additionally, ligation of either αVβ3 or αVβ5 may influence the function of the reciprocal receptor.

(Eliceiri et al., 1999) block VEGF- but not bFGF-induced angiogenesis. This elegant but simple initial model of growth factor–αV integrin coupling in angiogenesis has now evolved into a complicated picture of intricate interactions between growth factor receptors and integrins.

How do growth factors influence αV integrin–mediated function?

Although they share few structural similarities and recognize widely different ligands, growth factor receptors and integrins elicit overlapping and, in some cases, additive intracellular effects (Fig. 1). Synergy between integrins and growth factors may occur in signaling complexes that cluster along the cell surface (Plopper et al., 1995). Substantial evidence points to both a physical and functional association between integrins and VEGFR-2 that may be regulated by VEGF. VEGF-induced tyrosine phosphorylated of VEGFR-2 and cell proliferation is augmented in endothelial cells adherent to the αVβ3 ligand vitronectin (Soldi et al., 1999). After VEGF stimulation, tyrosine-phosphorylated VEGFR-2 coimmunoprecipitates with αVβ3 but not with integrin β1 or β5. Moreover, function-blocking antibodies to αV and β3 inhibit VEGF-stimulated phosphorylation of VEGFR-2 and activation of the regulatory subunit of phosphatidylinositol (PI) 3-kinase (Soldi et al., 1999). In CHO cells, VEGFR-2 immunoprecipitates with αVβ3 but not with integrin β5 (Borges et al., 2000) apparently through interactions involving the extracellular domain of integrin β3. Deletion or alteration of the β3 cytoplasmic domain does not affect the association (Borges et al., 2000), suggesting that the interaction does not require focal adhesion formation.

Growth factors modify the signals necessary for angiogenesis by altering the levels of integrins and their affinity for ligands. The normally low endothelial expression of αVβ3 (Brooks et al., 1994a) can be upregulated by bFGF (Enenstein et al., 1992; Brooks et al., 1994a; Sepp et al., 1994) and

VEGF (Senger et al., 1996) but not by TGFβ (Enenstein et al., 1992; Sepp et al., 1994). The effects of individual growth factors are integrin specific, since TGFβ heightens expression of the more abundant β1 integrins, whereas bFGF's effects are restricted to αVβ3. VEGF binding to VEGFR-2 activates multiple integrins, including αVβ3, αVβ5, α5β1, and α2β1, to enhance cell adhesion and migration (Byzova et al., 2000). Particular tumors with high adhesive properties display autocrine/paracrine integrin activation by VEGF (Byzova et al., 2000). Finally, by activating the small GTP-binding protein Rac, bFGF enhances Rac-dependent recruitment of activated αVβ3 to lamellipodia where the receptor directs cell migration (Kiosses et al., 2001).

What specific signaling pathways are coupled to αV integrins?

Integrins play complex roles in controlling cell migration, growth, differentiation, and apoptosis. Because of the redundancy in the matrix proteins that are recognized by αVβ3 and αVβ5, delineating their contribution to endothelial cell biology has relied in large part on the use of inhibitors or matrix molecules that appear to specifically target one or the other integrin. For example, Del1, an ECM protein and potent angiogenic factor whose expression is restricted to endothelial cells (Hidai et al., 1998), binds αVβ3 but not αVβ5 and triggers focal adhesion formation and phosphorylation of focal adhesion kinase (FAK), mitogen-activated protein kinase (MAPK) (extracellular-regulated kinase), and Shc (Penta et al., 1999). Arachidonic acid metabolism may also be critical to αVβ3-dependent endothelial migration and angiogenesis. Dormond et al. (2001) demonstrated recently that inhibition of cyclooxygenase-2 prevents αVβ3-mediated endothelial cell spreading, migration, and activation of Cdc 42 and Rac. These effects are overcome by prostaglandins, phorbol esters, and constitutively active Cdc42 or Rac, suggesting that an unidentified arachidonic acid metabolite may play a critical role in αVβ3-mediated activation of Rac. In an angiogenesis model, constitutively active Rac restored bFGF-induced angiogenesis in the presence of cyclooxygenase-2 inhibition. This exciting link between αVβ3, arachidonic acid metabolism, and angiogenesis provides a novel mechanistic explanation for the observation that nonsteroid antiinflammatory agents protect against cancer development and progression.

Several observations implicate αVβ3 in the control of cell survival and proliferation. Administration of αVβ3 antibody antagonists results in apoptosis of angiogenic but not quiescent vascular cells (Brooks et al., 1994b). Immobilization of endothelial cells on plates coated with αVβ3 antibodies suppresses p53 and the bax cell death pathway, and inhibition of αVβ3- but not αVβ5- or β1-mediated cell adhesion activates p53 (Strömblad et al., 1996). Furthermore, αV antagonists appear to require the presence of p53 to inhibit retinal neovascularization, in that p53-deficient mice are protected from their effects (Strömblad et al., 2002). NF-κB also plays an important role in αVβ3-mediated endothelial cell survival after serum deprivation (Scatena et al., 1998). Moreover, αVβ3 antagonists block sustained endothelial MAPK activity in bFGF-treated chick chorioallantoic membranes. (Eliceiri et al., 1998). A recent article demonstrated that endothelial αVβ3 elicits an "integrin-mediated death" pathway in cells grown in an environment devoid of αVβ3 ligands (Stupack et al., 2001). Unligated αVβ3 appears to initiate apoptosis by recruiting and activating caspase-8, an effect that is mimicked by the proximal regions of the cytoplasmic domains of both β3 and β1 but not β5. Limited calpain-dependent cleavage of the cytoplasmic domain of β3 has been observed early in the course of suspension-induced apoptosis in endothelial cells (Meredith et al., 1998). Whether calpain cleavage of β3 disrupts prosurvival signals generated by αVβ3 and/or facilitates the recruitment of caspase 8 in nonadherent endothelial cells remains to be determined.

Several endogenous angiogenesis inhibitors may exert their antiproliferative effects, in part, via αVβ3 (see Table I for a more complete list). Endothelial cell attachment to immobilized endostatin (a 20-kd collagen COOH terminus cleavage product) is mediated by αVβ3, α5β1, and αVβ5 (Rehn et al., 2001); adhesion to immobilized tumstatin (NC1 domain of the α3 chain of type IV collagen) is inhibited by antibodies to αVβ3, β1, and α6 (Maeshima et al., 2000). Both soluble endostatin and tumstatin inhibit endothelial cell proliferation, but endostatin elicits minimal apoptosis (2–5% cells), whereas soluble tumstatin and tumstatin peptide derivatives induce apoptosis at levels comparable to tumor necrosis factor–α (Maeshima et al., 2001). Tumstatin also prevents αVβ3-dependent activation of FAK, PI 3-kinase, protein kinase B (Akt), and cap-dependent protein synthesis in endothelial

Table I. **Endogenous angiogenic proteins/proteolytic fragments that may exert their effects, at least in part, via integrins**

Protein/proteolytic fragment	Effect	Integrin receptor(s)	Nonintegrin receptor(s)	Reference
Angiopoietins 1 and 2	Proangiogenic	α5β1	Tie-2	Carlson et al., 2001
ANGPTL3	Proangiogenic	αVβ3		Camenisch et al., 2002
Angiostatin	Antiangiogenic	αVβ3	ATP synthase, angiomotin	Tarui et al., 2001
Cysteine-rich 61 (CYR61, CCN1)	Proangiogenic/possible tumor suppressor	αVβ3, αVβ5, α6β1	Heparan sulfate proteoglycans	Grzeszkiewicz et al., 2001
Del1	Proangiogenic	αVβ3		Hidai et al., 1998
Endostatin	Antiangiogenic	αVβ3, αVβ5, α2β1, a5β1	Glypican (a heparan sulfate proteoglycan)	Rehn et al., 2001
Thrombospondin-1	Antiangiogenic	αVβ3, α3β1, α4β1, α5β1	CD36, CD47 (integrin-associated protein) Low density lipoprotein receptor–related protein Heparan sulfate proteoglycans	For review see Bornstein, 2001
Tumstatin	Antiangiogenic	αVβ3, α6β1		Maeshima et al., 2000

cells (Maeshima et al., 2002). The separate effects of endostatin and tumstatin on endothelial cell function may be mediated by distinct conformations assumed by αVβ3 upon binding these two ligands or may be the result of additional signals generated by integrins other than αVβ3 (e.g., αVβ5 in the case of endostatin). The interaction of fibroblasts with CYR61, an angiogenic matrix molecule that binds both αVβ5 and αVβ3, demonstrates that αV integrins are able to mediate discrete functions upon binding the same ligand: CYR61 engagement of αVβ5 promotes fibroblast migration, but engagement of αVβ3 is required to enhance bFGF-induced proliferation (Grzeszkiewicz et al., 2001).

Although αVβ3 appears to play a key role in regulating endothelial cell survival, proliferation, and apoptosis, αVβ5 has been linked to Src-dependent pathways stimulated by VEGF. In stroke models where VEGF contributes to cerebral vascular permeability, brain edema, and injury (van Bruggen et al., 1999), Src-deficient mice and wild-type mice treated with Src inhibitors display reduced vascular permeability and smaller infarct volumes (Paul et al., 2001). In a recent article, Eliceiri et al. (2002) demonstrated that mice deficient in integrin β5 are similarly protected from the effects of VEGF. More importantly, they elucidate the first mechanistic link between VEGF-stimulated Src activity and αVβ5 by demonstrating that VEGF promotes Src-dependent association of FAK with αVβ5 in endothelial cells. VEGF stimulation dramatically increases the coimmunoprecipitation of FAK with αVβ5, an event that involves Src-mediated phosphorylation of FAK at tyrosine residue 861. The effects are selective in that VEGF does not alter the constitutive association of αVβ3 and FAK, and bFGF does not promote αVβ5–FAK complex formation. These findings provide a novel mechanism for VEGF regulation of αVβ5 signaling, which places Src upstream rather than downstream of FAK activation and integrin association.

Clearly, αV integrin-dependent events do not occur in isolation, and other endothelial integrins may influence angiogenic events. Additionally, integrin cross-talk, the phenomena in which ligation of one integrin influences the behavior of a second integrin on the same cell, may regulate endothelial αVβ3 function. Antagonists of integrin α5β1, which block growth factor- and tumor-induced angiogenesis, inhibit αVβ3-promoted human umbilical vein endothelial cell migration and focal contact formation via a protein kinase A–dependent pathway (Kim et al., 2000). Interestingly, in the same cells αVβ3 antagonists have been shown to block α5β1-mediated migration (Simon et al., 1997), suggesting that endothelial integrin cross-talk may be bidirectional.

What has target gene deletion in mice revealed about the role of αV integrins in angiogenesis?

Studies in mice with targeted gene deletions of either αV, β3, or β5 were initially less informative than anticipated with regard to the role of these integrins in angiogenesis. Embryos deficient in αV develop normally until E9.5 and have unimpaired vasculogenesis and angiogenesis in many organs. Approximately 80% of the embryos die, apparently as the result of placental defects. The mice that survive suffer lethal intracranial and intestinal hemorrhage (Bader et al., 1998). Mice lacking either integrin β3 (Hodivala-Dilke et al., 1999) or β5 (Huang et al., 2000) undergo normal angiogenesis, and the pattern of retinal neovascularization in β3-null mice is indistinguishable from that in wild-type mice. In a follow-up to the original characterization of the $\beta3^{-/-}$ mice, Hodivala-Dilke's group reported that tumors grown in $\beta3^{-/-}$ mice or in mice with a combined deficiency of β3 and β5 are larger in size and display enhanced angiogenesis (Reynolds et al., 2002). They observed an augmented angiogenic response to VEGF in $\beta3^{-/-}$ endothelial cells that corresponded to increased levels of VEGFR-2, suggesting that upregulation of VEGF signaling may enhance tumor angiogenesis in β3-deficient mice. Both Eliceiri's latest work and recent studies in the $\beta3^{-/-}$ mice establish that a closer examination of the $\beta5^{-/-}$ and $\beta3^{-/-}$ mice is warranted and may reveal fascinating information about the relationship between integrins and growth factors.

Bringing together αV integrins, growth factors, and angiogenesis

A wealth of data indicates that growth factor receptors and αV integrins interact physically and functionally to generate the signals necessary for angiogenesis (Fig. 1). Many of the responses modulated by αVβ3 are linked to proliferative and/or apoptotic pathways, whereas Eliceiri et al. (2002) convincingly tie αVβ5 with pathways involving FAK and Src. However, several questions remain. The chief question is how to resolve the discrepancies in the antiangiogenic effects of antibody and small molecule αVβ3 and/or αVβ5 inhibitors with the apparent normal developmental angiogenesis and enhanced tumor angiogenesis in the β3- and combined β3/β5-deficient mice. Does compensatory upregulation of VEGFR-2 account for normal developmental angiogenesis in the $\beta3^{-/-}$ mice? In the absence of αVβ3, does VEGF enhance the affinity of alternate compensatory integrins? Or, do αVβ3 antagonists mediate their effects through distinct signaling mechanisms such as by recruitment of caspase 8 with subsequent activation of apoptotic pathways or by transdominant integrin inhibition? Do growth factors paradoxically induce endothelial cell susceptibility to αVβ3 antagonists by upregulating receptor levels? One hypothesis that reconciles the antiangiogenic effects of αV inhibitors with enhanced tumor angiogenesis in mice lacking αVβ3 is the receptor can either promote or inhibit endothelial cell survival/proliferation depending on the presence of external stimuli and the composition of the ECM. Thus, under certain conditions αVβ3 may assume a conformation that generates signals to maintain endothelial cells in a quiescent state. Tumor-induced alterations in growth factors and matrix may shift the conformation of, and signaling pathways generated by, αVβ3. In this scenario, the lack of basal αVβ3-mediated endothelial inhibition in $\beta3^{-/-}$ mice could result in enhanced proliferation in response to VEGF or other factors. Treatment with αV antagonists may maintain the initial αVβ3-mediated inhibitory signals and/or may trigger signals for apoptosis or transdominant inhibition of other integrins. Although investigations in this field have made rapid progress, the complexities of the integrin–growth factor nexus have not been fully revealed.

Due to space limitations, we were unable to cite all of the pertinent references. We apologize to those whose work was not included.

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