Published in final edited form as:
Cell Biochem Biophys. 2009 ; 53(2): 53–64. doi:10.1007/s12013-008-9040-5.

Integrin and Growth Factor Receptor Alliance in Angiogenesis

Payaningal R. Somanath, **Alieta Ciocea**, and **Tatiana V. Byzova**
Joseph J. Jacobs Center for Thrombosis and Vascular Biology, Department of Molecular Cardiology, NB50, Lerner Research Institute, The Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA

Tatiana V. Byzova: byzovat@ccf.org

Abstract

A sequence of events in vascular and stromal cells maintained in a highly coordinated manner regulates angiogenesis and tissue remodeling. These processes are mediated by the ability of cells to respond to environmental cues and activate surface integrins. Physiological and pathological processes in vascular biology are dependent on the specificity of important signaling mechanisms that are activated through the association between growth factors, their receptors, integrins, and their specific extracellular matrix ligands. A large body of evidence from in vitro and in vivo models demonstrates the importance of coordination of signals from the extracellular environment that activates specific tyrosine kinase receptors and integrins in order to regulate angiogenic processes in vivo. In addition to complex formation between growth factor receptors and integrins, growth factors and cytokines also directly interact with integrins, depending upon their concentration levels in the environment, and differentially regulate integrin-related processes. Recent studies from a number of laboratories including ours have provided important novel insights into the involvement of many signaling events that improve our existing knowledge on the cross-talk between growth factor receptors and integrins in the regulation of angiogenesis. In this review, our focus will be on updating the recent developments in the field of integrin-growth factor receptor associations and their implications in the vascular processes.

Introduction

Angiogenesis, the process of formation of new blood vessels from the pre-existing vasculature [1], is necessary for physiological processes, including embryogenesis, wound healing, and the normal response to hypoxic conditions and additionally underlies a number of pathologies, such as tumor development, rheumatoid arthritis, diabetic microvascular disease, macular degeneration, ischemia, and inflammation [2]. Development and remodeling of the vascular system and associated tissues require complex interactions of signals and physical forces orchestrating the activities of endothelial cells, pericytes, fibroblasts, and smooth muscle cells [3]. Endothelium, the vasculature's inner lining, is responsible for the initiation of angiogenesis, and crucial for this process is the ability of endothelium to attach to extracellular matrix (ECM) proteins and migrate to form endothelial tubes [4]. Formation of the initial endothelial tubes is followed by recruitment of stromal and inflammatory cells ultimately resulting in a network of matured blood vessels [4]. These series of events are regulated by a number of growth factors, cytokines, and ECM proteins and their receptors [5]. Initial endothelial tube formation requires the coordinated integration of mitogenic and migratory signals elicited by pro-angiogenic growth factors such as vascular endothelial growth factor

Correspondence to: Tatiana V. Byzova, byzovat@ccf.org.

(VEGF) [6]. Induction of endothelial cell migration by VEGF also depends on interactions between endothelium and various ECM proteins mediated by integrins [7]. Major integrins on endothelial cells include $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, and $\alpha_6\beta_4$, which serve as receptors for vitronectin, fibronectin, and laminin, respectively [7]. Among these, integrin $\alpha_v\beta_3$ has been shown to be particularly important for the angiogenic stimulation by VEGF and other growth factors [8–10].

In the last decade, an increasing body of evidences demonstrate that integrins are not mere ECM receptors, but major regulators of the biological activity of several signaling systems within the cell. A number of laboratories, including ours, have demonstrated that the specificity of molecular signaling in the endothelium during development and during pathological processes is determined by a synergism between growth factor receptors and cell adhesion molecules [11–16]. Integrins and growth factor receptors are known to independently regulate angiogenesis [17,18]. However, the combined effects of growth factors and ECM proteins via their respective receptors appear to be essential for endothelial activation and neovascularization [19,20]). For the better understanding of the molecular mechanisms underlying physiological and pathological angiogenesis, analysis of cross-talk between growth factor receptors and integrins with emphasis on the signaling cascades elicited by this interaction would be essential. Therefore, in this review, we will focus on the recent developments in our understanding of the interactions between growth factor receptors and integrins and the functional consequences of their synergism.

Integrins and ECM Interactions

Interactions between cells and ECM proteins are crucial for virtually all tissue responses, including cell movement, proliferation, and matrix remodeling [21]. Integrins represent that most important family of ECM receptors [7] and are found in all multicellular organisms ranging from sponges to mammals [22]. The biological significance of the range of ECM-integrin specificities during cell adhesion and migration remains an important issue. Integrins are present on the cell surface as heterodimers, each composed of non-homologous trans-membrane α and β subunits, both of which are involved in the recognition of ligands and regulate bi-directional signaling [7]. ‘Outside–in signaling’ provides the cells cues from the extracellular environment, while ‘inside–out signaling’ results in changes in the conformation of integrins, thus promoting modifications in their functional activity [23]. Vascular cells, which include endothelial cells, pericytes, fibroblasts, and smooth muscle cells, express numerous integrins including $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_6\beta_1$, and $\alpha_6\beta_4$ [7] and, therefore, are able to interact with a wide range of ligands. Thus, integrins have been widely implicated in the regulation of angiogenesis [24].

Changes in ECM environment result in the induction of multiple cellular signaling pathways in vascular cells via integrins [25]. These include activation of Ras, PI3 Kinase-Akt signaling, MAP kinase, Src and Rac, Rho, cdc42 GTPases [26–29]. Furthermore, recognition of ECM by some integrins such as $\alpha_v\beta_3$ results in the phosphorylation of integrin cytoplasmic tyrosine residues, which, in turn, promotes recruitment of intracellular adaptor proteins [15,30].

In many instances, activation of the intracellular signaling cascade triggered by growth factors is substantially amplified if endothelial cells are attached to ECM [31]. This clearly indicates that integrin- and growth factor-mediated cellular responses synergize and function to coordinate the biochemical responses of multiple cell types.

Integrin and Growth Factor Receptor Cross-Talk

A role of growth factors and their tyrosine kinase receptors in integrin-dependent processes such as ECM recognition, migration, matrix assembly, tissue remodeling, and angiogenesis is

gradually emerging. Optimal cell stimulation with epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin like growth factor-1 (IGF-1), or VEGF [15,32–34], is greatly influenced by integrin-mediated cell adhesion to the respective ECM protein. In vascular smooth muscle cells, EGF or IGF-1 responses depend on integrin $\alpha_v\beta_3$ [35–37], whereas functions of EGF-stimulated kidney epithelial cells depends on β_1 integrins [33]. The physiological importance of IGF-1/$\alpha_v\beta_3$ association is further supported by the fact that treatment with $\alpha_v\beta_3$ antagonists reduce IGF-1 signaling and development of atherosclerotic lesions [38,39]. Detailed information on interaction between growth factor receptors and integrins is provided in Table 1.

The capacity of growth factor stimulation to synergize with the ECM ligand associated signals may be due to the co-clustering of these receptors on the cell surface. Co-immunoprecipitation has been an important approach to identify physical interactions between growth factor receptors and integrins in vascular cells. Integrin $\alpha_v\beta_3$ has been reported to interact with the PDGF receptor (PDGFR) and VEGFR-2 [13,15,40,41]; as well as IRS-1, a cyto-plasmic signal transduction mediator of insulin and IGF receptors [42]. Additionally, integrins $\alpha_6\beta_4$ and $\alpha_6\beta_1$ interact with ErB-2 receptor in human breast carcinoma epithelial cells upon EGF stimulation [43,44]. An integrin-activating anti-α_6 antibody promotes association between $\alpha_6\beta_4$ and ErB-2, which, in turn, might be responsible for enhanced cell proliferation and invasion as a result of stimulation by the antibody [44]. The most recent data also indicate that integrin $\alpha_6\beta_4$ induces ErB-3 expression in breast carcinoma cells [45].

VEGF and VEGF Receptors

The mitogen VEGF regulates key steps in the initiation of angiogenesis such as endothelial cell activation, vascular permeability, trans-migration of inflammatory cells, endothelial migration, and proliferation [46]. VEGFs are a family of secreted polypeptides which, similar to PDGF, has a highly conserved cysteine-knot structure that is responsible for receptor binding. VEGF-A, the founder member of the family, is generally described as VEGF or vascular permeability factor (VPF). Endothelial responses by VEGF are exerted through binding to two homologous membrane tyrosine kinase receptors present on the surface of endothelial cells: VEGFR-1, encoded by the gene *Flt1*, and VEGFR-2, encoded by *Flk1/KDR* [47]. On endothelial cells, neuropilin is another receptor for VEGF that increases the affinity of VEGFR-2 for VEGF [48]. Gene knockout of VEGFR-1 and VEGFR-2 in mice results in lethality [49,50] indicating that these receptors are essential for the angiogenesis process during embryogenesis. A recent study also shows that partial knockdown of VEGFR-1 regulates excessive angiogenesis in response to VEGFR-2 activation [51].

Intrinsic tyrosine kinase activity in VEGFRs is triggered by ligand-induced homodimerization and oligomerization. Although reports suggest the formation of heterodimers between VEGFR-1 and VEGFR-2 [6,52], its role in endothelial cells is still ambiguous. Although VEGFR-1 knockout in mice results in embryonic lethality [49], knockouts expressing VEGFR-1 lacking its kinase domain possesses normal vasculature [53]. In contrast, strong phosphorylation of tyrosine residues in VEGFR-2 has been observed upon homodimerization [54]. Residues Y1175 and Y1214 have been described as major autophosphorylation sites on VEGFR-2 [55]. Other residues such as Y951 and Y996 in the kinase insert domain and Y1054 and Y1059 in the kinase catalytic domain [53] are putative phosphorylation sites that may be important for VEGFR-2's interaction with other molecules or receptors.

Recent studies demonstrate that the role of VEGFR-1 in postnatal angiogenesis is more complicated than initially recognized. Treatment with placental growth factor (PlGF), a VEGFR-1 specific ligand, promotes angiogenesis in vitro and in vivo [56]. Over-expression of PlGF also results in enhanced angiogenesis in tumor and skin [57]. It has been reported that

PlGF stimulates oligomerization of VEGFR-1 and VEGFR-2, leading to trans-activation of VEGFR-2 and promotion of angiogenesis [56]. Meantime, over-expression of PlGF in many cancer cell lines inhibited angiogenesis and tumor growth via inhibition of homodimerization of VEGFR-2 [58]. A recent study also shows that partial knockdown of VEGFR-1 regulates excessive angiogenesis in response to VEGFR-2 activation [51].

Integrin $\alpha_v\beta_3$ and VEGFR-2 Association

During initiation of angiogenesis, endothelial cells sprouting out of existing blood vessels adhere to a provisional ECM rich in vitronectin and fibrinogen via integrin $\alpha_v\beta_3$ [15,59]. Stimulation of endothelial cells with VEGF results in the formation of a complex between VEGFR-2 and integrin $\alpha_v\beta_3$ [15]. No similar effect was observed for collagen receptor $\alpha_2\beta_1$ and laminin receptors $\alpha_6\beta_1$ and $\alpha_6\beta_4$ [19,20]. Moreover, collagen, a ligand for $\alpha_2\beta_1$ and $\alpha_1\beta_1$ integrins, exerted an inhibitory effect on VEGFR-2 [60]. Endothelial cell adhesion to collagen reduced VEGF-induced VEGFR-2 phosphorylation by recruiting tyrosine phosphatase SHP2 to the phosphorylated tyrosine 1117 residue of VEGFR-2. One of the key functional consequences of an interaction between $\alpha_v\beta_3$ and VEGFR-2 is augmentation of integrin $\alpha_v\beta_3$ activity or its activation.

An anti-β_3 monoclonal antibody BV4, which inhibits adhesion to vitronectin (prototypic ligand for $\alpha_v\beta_3$) decreased phosphorylation of VEGFR-2 suggesting an important function of $\alpha_v\beta_3$ in the activation of the angiogenic program in endothelial cells [34]. In general, the blockade of $\alpha_v\beta_3$ results in diminished autophosphorylation of VEGFR-2 while integrin activation with LIBS-like activating antibodies or engagement by natural ligand promotes VEGFR-2 phosphorylation [13]. Our recent study showed that VEGFR-2 forms a complex with β_3 integrin subunit, but not with β_1 or β_5, when stimulated with VEGF [13,14] (Fig. 1). This complex can be observed in vivo as well as in cell culture [13]. Stimulation of endothelial cells by VEGF in the presence of ECM proteins induces phosphorylation of the β_3 cytoplasmic tyrosine residues, which, in turn, is required for association between VEGFR-2 and β_3 [14]. Mutations of both tyrosines within $\alpha_v\beta_3$ cytoplasmic domain to phenylalanine abolish the complex formation between $\alpha_v\beta_3$ and VEGFR-2 resulting in diminished VEGFR-2 activation by VEGF [14]. Other studies demonstrate that the extracellular but not the cytoplasmic domain of VEGFR-2 is crucial for integrin-VEGFR-2 cross signaling [32]. Although the structural details of the complex between $\alpha_v\beta_3$ and VEGFR-2 remain unclear, its formation might be dependent on both the extracellular and cytoplasmic domains of the β_3 subunit [14,15,34].

Once engaged by multivalent or immobilized ligands such as fibrin or fibrinogen, integrin $\alpha_v\beta_3$ is able to form clusters on the cell surface, an ability important for recruitment of additional components of this multireceptor complex that include VEGFR-2 and PI3K [61,62]. Illustration of the impact of $\alpha_v\beta_3$ integrin clustering on this complex was demonstrated using anti-β_3 antibody, which interferes with $\alpha_v\beta_3$ clustering but not with cell adhesion to ECM [63]. In addition to perturbing the formation of the complex, this antibody markedly inhibited VEGFR-2-mediated phosphorylation, PI3 kinase activation, focal adhesion dynamics, and migration of endothelial cells in response to VEGF. And vice versa, the clustering of integrins might promote certain cellular responses [61]. The complex between VEGFR-2 and $\alpha_v\beta_3$ has also been implicated in mediating the pro-angiogenic effects of Factor XIII (FXIII) of the coagulation cascade [64]. This activated transglutaminase crosslinks integrin β_3 to VEG-FR-2, thus enhancing their interaction and resulting in complex formation [65]. Interestingly, VEGFR-2 is not the only VEGF receptor known to interact with integrins. Stimulation of VEGFR-3 by VEGF-C or VEGF-D results in selective association between VEGFR-3 and integrin $\alpha_5\beta_1$ in lymphatic endothelial cells and this interaction appears to be involved in the regulation of lymphangiogenesis [66,67].

Integrin Growth Factor Receptor Cross-Talk Analysis with Knockout Mouse Models

Research during the last decade has resulted in the development and characterization of a number of knockout mouse models related to cardiovascular biology. This has provided important insights into the importance of integrins and growth factor receptors, associating to transduce downstream signals to regulate vascular development in a number of angiogenesis dependent processes. For example, in mice the lack of fibronectin or integrin $\alpha_5\beta_1$ results in embryonic lethality, indicating the importance of this integrin and its ligand in embryonic vasculogenesis [68]. This was further supported by studies involving gene ablation of integrin α_5 in mice, where endothelial structure formation is impaired in embryoid bodies [68,69]. In combination, these studies indicate the importance of fibronectin receptor in embryonic angiogenesis. In contrast, although integrin α_v gene knockout in mice is lethal [70], ablation of integrins β_5 and/or β_3 results in normal development of embryo and normal postnatal angiogenesis [71]. Both integrins β_5 and/or β_3 null mice have extensive tumor induced angiogenesis in vivo [71]. Since much of the other vasculature development appears to be normal, it clearly indicates that a compensatory mechanism does exist in these knockout mice. Endothelial cells in integrins β_5 and/ or β_3 mouse vasculature express higher amounts of VEG-FR-2, thus demonstrating a possible compensatory mechanism. In addition, this effect also indicates a close association between VEGFR-2 and integrin $\alpha_v\beta_3$ in endothelial cell activation and angiogenesis. A true function of integrin $\alpha_v\beta_3$ has been revealed by a knockin mouse model (DiYF mice) with two integrin β_3 cytoplasmic tyrosine residues mutated to phenylalanine (see below) [14]. In this case, pathological angiogenesis induced by tumor implantation is significantly reduced in DiYF mice compared to WT. Besides this, study also reveals that mutations in two integrin β_3 cytoplasmic tyrosine residues block the formation of complex between VEGFR-2 and integrin upon stimulation with VEGF. Another study reports that although mice lacking integrin β_5 develop normal vasculature, these mice have specific VEGF-induced vascular permeability defects [72]. Collectively, studies in these transgenic mice clearly demonstrate the importance of association between VEGFR-2 and integrin $\alpha_v\beta_3$ or integrin $\alpha_v\beta_5$ in VEGF induced angiogenic responses.

Role of Src Family of Kinases in Integrin Growth Factor Receptor Association

The Src family of kinases is a group of non-receptor tyrosine kinases that are activated both by activation of growth factor receptors and integrins through ‘outside–in signaling’ [73]. Major members of this family include cSrc, Fyn, Lyn, and Yes [74]. Many previous reports have suggested the Src family of kinases as potential regulators of integrin-growth factor receptor association in vascular cells [75]. Src has been reported to interact with EGFR to regulate cell proliferation [76], and in association with PDGFR, it induces integrin dependent cell adhesion and migration [77]. A functional overlap between the members in the same cell types is evident from specific knockout studies in mice. Although cSrc null mice develop normal blood vessels, they exhibit osteoporosis and impaired vascular permeability in response to VEGF [78,79]. Interestingly, mice deficient in Yes also exhibit defects in VEGF induced vascular permeability [80], suggesting non-redundant functions of these two Src kinases in the regulation of vascular permeability. In contrast, Fyn-deficient mice do not exhibit a defect in vascular leakage induced by VEGF [81]. Mice lacking Src kinases cSrc, Yes, and Fyn (SYF) develop blood-filled islands in the embryo leading to lethality [82]. These reports demonstrate that, if not all, many of the functional requirements of the Src family of tyrosine kinases are compensated for in the absence of one. However, together, they are absolutely necessary for the normal development of embryonic and post-natal vasculature.

A recent study in our lab has demonstrated the non-redundant function of cSrc in vascular cells in the regulation of integrin $\alpha_v\beta_3$ and VEGFR-2 complex formation via inside–out signaling

[15]. Phosphorylation by VEGF stimulation of both Y747 and Y759 in the integrin β_3 cytoplasmic domain was observed to be enhanced when endothelial cells are plated on vitronectin compared to other ECM proteins. Antibodies that specifically block the function of α_v or β_3, but not β_1 or β_5, inhibited VEGF stimulated tyrosine phosphorylation of both integrin β_3 and VEGFR-2. Immunoprecipitation analysis revealed that stimulation with VEGF results in complex formation between VEGFR-2 and integrin β_3 and not with β_1 or β_5 [13]. Treatment with Src inhibitors completely blocked VEGF mediated complex formation between VEGFR-2 and integrin β_3 (Fig. 1). At basal activity, only Yes is associated with integrin β_3 and treatment with VEGF had no effect on modulating its interaction with integrin β_3. In contrast, cSrc interaction with integrin β_3 was enhanced upon VEGF stimulation. Fyn did not interact with integrin β_3 in either of the conditions. These data suggested the non-overlapping role of cSrc in the regulation of VEGFR-2 and integrin β_3 association.

Further analysis revealed the importance of Src kinases in the phosphorylation of integrin β_3 cytoplasmic tyrosine residues [13]. Phosphorylation of integrin β_3 was significantly reduced in SYF null mice. While treatment with Src inhibitor or expression with dominant negative cSrc inhibited phosphorylation of integrin β_3 cytoplasmic tyrosine residues, expression with the constitutively active form of cSrc resulted in enhanced phosphorylation of both Y747 and Y759. Most importantly, in vitro phosphorylation assay demonstrated that recombinant cSrc phosphorylates integrin β_3 at the cytoplasmic tyrosine residues. Specific inactivation of cSrc results in inhibition of complex formation between VEGFR-2 and integrin β_3 resulting in impaired binding to the specific $\alpha_v\beta_3$ ligand vitronectin, but not to other integrin ligands like collagen and laminin. These facts point out the direct and specific involvement of c-Src in the regulation of $\alpha_v\beta_3$ interaction with ECM. These induced structural changes caused by β_3 phosphorylation promoted signaling that resulted in pre-capillary tube formation and enhanced chemotaxis upon VEGF stimulation.

Integrin $\alpha_v\beta_3$ and VEGFR-2 Cross-Talk Analysis with DiYF Transgenic Mice

Studies demonstrating the importance of cSrc in regulating the association between VEGFR-2 and integrin β_3 indicated the importance of integrin β_3 tyrosine residues in their complex formation. This report was further strengthened by the data derived from knockin mice, where the two tyrosine residues Y747 and Y759 are mutated to phenylalanine [14]. In the DiYF (two Y to F mutations in the integrin β_3 cytoplasmic domain) knockin mouse model, phosphorylation of VEGFR-2 in response to VEGF is impaired. DiYF mutations in integrin β_3 cytoplasmic domain also resulted in the blockade of complex formation between integrin β_3 and VEGFR-2.

Reduced interaction between VEGFR-2 and integrin β_3, impaired VEGF signaling and reduced VEGFR-2 phosphorylation in DiYF endothelial cells resulted in their impaired adhesion and migration on vitronectin and entactin, two major $\alpha_v\beta_3$ ligands in the tissues and basement membrane, respectively [83]. In vivo, tumor angiogenesis was impaired in DiYF mice resulting in reduced tumor growth [14]. Overall, these studies in DiYF knockin mice reveal the importance of the two tyrosine residues in integrin β_3 in the formation of complex with VEGFR-2, which then results in conformational activation via inside–out signaling, which enhances its affinity for the ECM substrate, vitronectin.

Integrin and FGF Receptor Association

Interaction between VEGFR-2 and $\alpha_v\beta_3$ integrin regulates VEGF signaling and interactions of endothelial cells with vitronectin, fibrin, and entactin/nidogen [14,84]. However, pro-angiogenic activities are not limited to VEGF and these selected components of matrix. Originally, it had been shown that neutralizing antibody against $\alpha_v\beta_3$ integrin diminished bFGF stimulated vascular survival and endothelial cell migration, suggesting the cooperation

between FGF-RTK and integrin $\alpha_v\beta_3$ in angiogenesis [85]. Also, similar to VEGF, stimulation of endothelial cells with bFGF might also promote activation of $\alpha_v\beta_3$ [59]. However, the molecular mechanisms underlying the cooperation between these receptors are not yet elucidated, with the exception of a study showing the importance of endothelial cell engagement by fibrinogen during complex formation of FGF-RTK and $\alpha_v\beta_3$ upon bFGF stimulation [86]. In addition, FGF receptor-3 has also been implicated to interact with various types of integrins [87].

Integrin and Tie-2 Association

Besides VEGFR and FGF-R, a third major receptor tyrosine kinase that has been implicated in the regulation of endothelial function and angiogenesis is Tie-2 receptor, which binds to its ligands Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) [88]. Both angiopoietins interact with the same receptor, Tie-2, and reciprocally regulate vascular stability [89]. While Ang-1 is essential for the maturation of blood vessels and reduction in vascular permeability, thereby stabilizing the newly formed blood vessels [90], Ang-2, competes with Ang-1 for the Tie-2 receptor and enhances vascular permeability and destabilization of blood vessels [91], which is a prerequisite for VEGF mediated vascular permeability and extravasation [92].

A recent report suggests that in order for Ang-1 to regulate vascular stabilization, its receptor Tie-2 has to be associated with integrin $\alpha_5\beta_1$ [93] (Fig. 2). Engagement of integrin $\alpha_5\beta_1$ by fibronectin increases integrin interaction with Tie-2 in a time and concentration dependent manner. This results in Tie-2 phosphorylation in response to threshold levels of Ang-1. While in complex with integrin $\alpha_5\beta_1$, Tie-2 activity is sustained for longer than an hour compared to transient effects upon Ang-1 stimulation in the absence of fibronectin. Thus, similar to $\alpha_v\beta_3$ and VEGFR-2, there is a functional alliance between integrin $\alpha_5\beta_1$ and Tie-2 receptor (Fig. 2). In endothelial cells attached to fibronectin, Ang-1 stimulates recruitment of p85 to Tie-2 [88] and FAK to the cytoplasmic tails of integrin $\alpha_5\beta_1$ [41] within focal adhesions. This complex formation between Tie-2 and integrin $\alpha_5\beta_1$ appears to involve the activation of PI-3 kinase signaling downstream of FAK activation [88,94]. In addition to its interaction with integrin $\alpha_5\beta_1$, FAK is also reported to regulate vascular permeability in response to VEGF activation involving integrin $\alpha_v\beta_5$ [95]. Moreover, another report shows that Ang-1 can directly bind to integrin $\alpha_5\beta_1$ even in the absence of Tie-2 receptor [93]. Overall, these studies show that Tie-2 and integrin $\alpha_5\beta_1$ form complexes in order to regulate vascular stabilization.

Integrins and Met Association

In addition to the major tyrosine kinase receptors discussed above, angiogenesis is also under the tight control of many pleiotropic molecules such as hepatocyte growth factor (HGF), which interacts with and activates Met, a receptor tyrosine kinase [96]. A number of in vitro and in vivo models demonstrate that HGF activates Met on the endothelial cell surface and promotes angiogenesis [97]. Importantly, HGF regulates matrix recognition by endothelial integrins [98]. HGF, largely synthesized and secreted by platelets, forms hetero-complexes with vitronectin and fibronectin, two important ligands for integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$ [99]. These hetero-complexes, VN/HGF and FN/HGF, trigger association between Met and integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$, respectively [98] (Fig. 3). As a result, autophosphorylation of Met in the presence of ECM is enhanced compared to that in the absence of VN or FN. Moreover, another report demonstrates that complex formation between Met and integrin $\alpha_6\beta_4$, a laminin receptor [100], is necessary for the regulation of tumor angiogenesis [101], thus demonstrating the importance of Met/integrin $\alpha_6\beta_4$ association in pathological angiogenesis.

Regulation of Integrin Function by Semaphorins

Semaphorins (Sema) are a family of secreted and membrane bound cytokines that signal through four classes of plexins (type A–D), a family of membrane receptors characterized by the presence in their cytosolic tail, two domains with homology to the R-Ras GTPase activating proteins (GAPs), separated by a linker region that can bind other small GTPases, such as Rnd-1 and Rac1 [19,20]. Sema of secreted class 3 group also employ Neurophilin (Nrp-1 or -2) as co-receptors in association with type A or type D plexins [19,20]. Nrp-1 was initially shown to act as a VEGFR-2 co-receptor with implications in cardiovascular development [48,102–104]. Observations in vitro and in vivo that endothelial cells exhibit autocrine loops of several Sema 3 other than Sema 3A [105], suggest that multiple Sema 3 could cooperate to regulate angiogenesis. In endothelial cells, plexinD1 and to a lesser extend plexinA2, are the most abundant plexins, which possesses significantly higher affinities for Sema3A and Sema3C [106].

At the level of endothelial cells, both Sema3A and Sema3F have shown to inhibit adhesion and migration via inhibition of integrins [107]. This was later confirmed in other cell types as well [108]. In contrast, Sema3C, which possesses an Arg-Gly-Asp (RGD) motif, has been reported to serve as a ligand for integrins and promote endothelial cell adhesion and migration [109]. Endothelial cell adhesion and migration is also regulated by Sema4D via PlexinB1 stimulation and suppression of R-Ras activity, which in turn inhibits integrin β_1 activation [110]. Sema4D thus promotes association between PlexinB1 and Met tyrosine kinase receptor and prevents interaction between integrin β_1 and Met [111]. Collectively, these reports suggest that semaphorins, via interaction with multiple growth factors, might inhibit growth factor stimulated activation of integrins and cross-talk.

Integrins and their Interactions with Angiogenic Modulators

The complex ECM, which has a very high affinity for many soluble growth factors, has been demonstrated to act as sequestering machinery for growth factors such as TGFβ [112]. Recent reports demonstrate that many pro- and anti-angiogenic molecules may be entrapped in the ECM and be directly recognized by integrins. Integrin $\alpha_9\beta_1$ has been shown to recognize immobilized VEGF-A165 and VEGF-A121 [113,114]. In response to these immobilized growth factors, VEGF, VEGFR-2, and integrin $\alpha_9\beta_1$ form a complex and transduce signals in an adaptive manner [114]. The same integrin had later been shown to interact with VEGF-C, VEGF-D, and HGF, and the lack of these interactions might explain the abnormal lymphatic development in integrin α_9 knockout mice [113]. Additionally, integrins $\alpha_3\beta_1$ and $\alpha_v\beta_3$ have been reported to interact with VEGF-A165 and VEGF-A189, but not with VEGF-A121 [115]. A recent report indicates that bFGF might also directly bind to integrin $\alpha_v\beta_3$ and regulate bFGF-induced endothelial activation and angiogenesis involving ERK and Akt activation [116]. Detailed information on interaction between angiogenic modulators and integrins is provided in Table 2.

Integrins are also known to partially mediate pro-angiogenic effects of Ang-1 and Ang-2 via direct interactions [117]. The region of Ang-1 and -2 that binds to integrins is located within the fibrinogen-like domain (QHREDGS), which resembles the integrin recognition motifs KRLDGS or REDV of fibrinogen and fibronectin, respectively [93,118–120]. Both endothelial cells and fibroblasts adhere to Ang-1 and Ang-2. However, only Ang-2 is able to promote cell spreading and cytoskeletal remodeling [118]. Ang-1 has been reported to serve as an important ligand for integrin $\alpha_5\beta_1$ in the absence of Tie-2, a well characterized receptor for both Ang-1 and -2 [118]. These observations combined with the cooperation between Tie-2 and integrin $\alpha_5\beta_1$ discussed above indicate that Ang-1 has the potential to trigger both inside–out and outside–in signals. In many cell types lacking Tie-2, Ang-1 and -2 interact directly with

integrins. In cardiomyocytes integrins $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, $\alpha_v\beta_1$, and $\alpha_v\beta_3$ have been shown to bind to Ang-1, resulting in Akt activation and cell survival [121]. Glioma cells have also been reported to attach to Ang-2 using many of these receptors, with the exception for $\alpha_v\beta_1$ [120]. Breast cancer epithelial cells bind to Ang-2 and promote migration involving integrin $\alpha_5\beta_1$ [122]. Altogether, these reports indicate that direct interaction between integrins and Ang-1 and -2 modulate the process of angiogenesis.

Proteolytic degradation of ECM results in the release of a number of anti-angiogenic molecules that have the ability to bind and inactivate endothelial integrins, thus resulting in inhibition of endothelial tube formation and angiogenesis [123]. While ECM ligands interact with and activate integrins on the endothelial cell surface, thus protecting the cells from anoikis [124], integrins engaged by the ECM fragments after proteolytic degradation induce apoptosis and inhibit proliferation and migration of endothelial cells [41]. One such molecule is endostatin, which is a C-terminal non-collagenous domain of type XVIII collagen that exerts inhibitory effects on integrin $\alpha_5\beta_1$ through an Argrich peptide at its N-terminus [125]. Proteolytic degradation of non-collagenous domain of the α-chain of type IV collagen results in three different anti-angiogenic molecules namely tumstatin, arresten, and canstatin [126]. Among these, tumstatin binds to integrin $\alpha_v\beta_3$ and promotes apoptosis, as a result of inhibition of FAK, Akt, and mTOR signaling [127,128]. Arresten, generated from the non-collagenous domain of the α1 chain of type IV collagen, competes with collagen IV binding to integrin $\alpha_1\beta_1$ thus inhibiting endothelial cell interaction with collagen and vitronectin [21,129]. Canstatin, derived from the α2 chain of type IV collagen, binds to integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ resulting in endothelial cell activation of caspases and promotion of endothelial cell apoptosis [130]. Altogether, these reports indicate that ECM can promote and inhibit endothelial integrin activity, which is dependent on its intact or fragmented nature.

Angiostatin is another known anti-angiogenic molecule that is proteolytically derived internal fragment of serine protease plasminogen. This contains various members of the five plasminogen “kringle” domains, depending on the sites of proteolysis. Different forms of angiostatin exhibit different activities. A number of groups have sought to identify the native cell surface binding site(s) for angiostatin, resulting in at least five different binding sites proposed for angiostatin on the surface of endothelial cells [131]. Angiostatins have been reported to bind to integrin $\alpha_v\beta_3$ thereby inhibiting angiogenesis [132].

Conclusions

Anti-angiogenic therapy is emerging as an effective tool for the treatment of many different types of cancers. Modulation of angiogenesis can also be useful for other angiogenesis-dependent anomalies such as ischemia, myocardial infarction, and post-surgery wound complications as well as physiological processes such as embryogenesis. These associations that regulate the assembly and remodeling of ECM by the vascular cells are also necessary for the integrity and remodeling of injured tissue. Many of the current day methods for targeting angiogenesis are via inhibitors and monoclonal antibodies against integrins, growth factors and growth factor receptors. Many of these therapeutic approaches have suffered major setbacks, mainly due to the uncertainties in the complex signaling networks these receptors regulate in vascular cells and the injured tissue. A complete analysis of the signaling pathways regulating the complex association between multiple growth factors, their receptors and integrins will be extremely useful in order to improve the existing methods of targeting angiogenesis involving these receptors. Hence, further validation of existing hypotheses in the mechanistic aspects of integrin-growth factor receptor association by the next generation of researchers is essential.

References

1. Folkman J. Angiogenesis: An organizing principle for drug discovery? Nature Reviews. Drug Discovery 2007;6(4):273–286.
2. Folkman J. Angiogenesis. Annual Review of Medicine 2006;57:1–18.
3. Gerhardt H, Betsholtz C. Endothelial-pericyte interactions in angiogenesis. Cell and Tissue Research 2003;314(1):15–23. [PubMed: 12883993]
4. Davis GE, Senger DR. Endothelial extracellular matrix: Biosynthesis, remodeling, and functions during vascular morphogenesis and neovessel stabilization. Circulation Research 2005;97(11):1093–1107. [PubMed: 16306453]
5. Napione L, Cascone I, et al. Integrins: A flexible platform for endothelial vascular tyrosine kinase receptors. Autoimmunity Reviews 2007;7(1):18–22. [PubMed: 17967720]
6. Rousseau S, Houle F, et al. Integrating the VEGF signals leading to actin-based motility in vascular endothelial cells. Trends in Cardiovascular Medicine 2000;10(8):321–327. [PubMed: 11369257]
7. Plow EF, Haas TA, et al. Ligand binding to integrins. The Journal of Biological Chemistry 2000;275 (29):21785–21788. [PubMed: 10801897]
8. Byzova TV, Goldman CK, et al. Adenovirus encoding vascular endothelial growth factor-D induces tissue-specific vascular patterns in vivo. Blood 2002;99(12):4434–4442. [PubMed: 12036873]
9. Byzova TV, Rabbani R, et al. Role of integrin alpha(v)beta3 in vascular biology. Thrombosis and Haemostasis 1998;80(5):726–734. [PubMed: 9843163]
10. Cheresh DA. Structure, function and biological properties of integrin alpha v beta 3 on human melanoma cells. Cancer Metastasis Reviews 1991;10(1):3–10. [PubMed: 1717171]
11. Brakenhielm E. Substrate matters: Reciprocally stimulatory integrin and VEGF signaling in endothelial cells. Circulation Research 2007;101(6):536–538. [PubMed: 17872470]
12. Mahabeleshwar GH, Byzova TV. Angiogenesis in melanoma. Seminars in Oncology 2007;34(6): 555–565. [PubMed: 18083379]
13. Mahabeleshwar GH, Chen J, et al. Integrin affinity modulation in angiogenesis. Cell Cycle 2008;7 (3):335–347. [PubMed: 18287811]
14. Mahabeleshwar GH, Feng W, et al. Integrin signaling is critical for pathological angiogenesis. Journal of Experimental Medicine 2006;203(11):2495–2507. [PubMed: 17030947]
15. Mahabeleshwar GH, Feng W, et al. Mechanisms of integrin-vascular endothelial growth factor receptor cross-activation in angiogenesis. Circulation Research 2007;101(6):570–580. [PubMed: 17641225]
16. Ogita H, Takai Y. Cross-talk among integrin, cadherin, and growth factor receptor: Roles of nectin and nectin-like molecule. International Review of Cytology 2008;265:1–54. [PubMed: 18275885]
17. Naik TU, Naik MU, et al. Junctional adhesion molecules in angiogenesis. Frontiers in Bioscience: A Journal and Virtual Library 2008;13:258–262. [PubMed: 17981544]
18. Hofer E, Schweighofer B. Signal transduction induced in endothelial cells by growth factor receptors involved in angiogenesis. Thrombosis and Haemostasis 2007;97(3):355–363. [PubMed: 17334501]
19. Serini G, Napione L, et al. Besides adhesion: New perspectives of integrin functions in angiogenesis. Cardiovascular Research 2008;78(2):213–222. [PubMed: 18285512]
20. Serini G, Napione L, et al. Integrins team up with tyrosine kinase receptors and plexins to control angiogenesis. Current Opinion in Hematology 2008;15(3):235–242. [PubMed: 18391791]
21. Suh DY. Understanding angiogenesis and its clinical applications. Annals of Clinical and Laboratory Science 2000;30(3):227–238. [PubMed: 10945562]
22. Muller WE, Muller IM. Analysis of the sponge [Porifera] gene repertoire: Implications for the evolution of the metazoan body plan. Progress in Molecular and Subcellular Biology 2003;37:1–33. [PubMed: 15825638]
23. Luo BH, Carman CV, et al. Structural basis of integrin regulation and signaling. Annual Review of Immunology 2007;25:619–647.
24. Stupack DG, Cheresh DA. Integrins and angiogenesis. Current Topics in Developmental Biology 2004;64:207–238. [PubMed: 15563949]

25. Michel JB. Anoikis in the cardiovascular system: Known and unknown extracellular mediators. Arteriosclerosis, Thrombosis, and Vascular Biology 2003;23(12):2146–2154.
26. Bryan BA, D'Amore PA. What tangled webs they weave: Rho-GTPase control of angiogenesis. Cellular and Molecular Life Sciences 2007;64(16):2053–2065. [PubMed: 17530172]
27. Kanda S, Miyata Y, et al. Non-receptor protein-tyrosine kinases as molecular targets for antiangiogenic therapy (Review). International Journal of Molecular Medicine 2007;20(1):113–121. [PubMed: 17549397]
28. Pouyssegur J, Volmat V, et al. Fidelity and spatio-temporal control in MAP kinase (ERKs) signalling. Biochemical Pharmacology 2002;64(5–6):755–763. [PubMed: 12213567]
29. Somanath PR, Razorenova OV, et al. Akt1 in endothelial cell and angiogenesis. Cell Cycle 2006;5 (5):512–518. [PubMed: 16552185]
30. Phillips DR, Nannizzi-Alaimo L, et al. Beta3 tyrosine phosphorylation in alphaIIbbeta3 (platelet membrane GP IIb-IIIa) outside-in integrin signaling. Thrombosis and Haemostasis 2001;86(1):246–258. [PubMed: 11487013]
31. Somanath PR, Kandel ES, et al. Akt1 signaling regulates integrin activation, matrix recognition, and fibronectin assembly. The Journal of Biological Chemistry 2007;282(31):22964–22976. [PubMed: 17562714]
32. Borges E, Jan Y, et al. Platelet-derived growth factor receptor beta and vascular endothelial growth factor receptor 2 bind to the beta 3 integrin through its extracellular domain. The Journal of Biological Chemistry 2000;275(51):39867–39873. [PubMed: 10964931]
33. Cybulsky AV, McTavish AJ, et al. Extracellular matrix modulates epidermal growth factor receptor activation in rat glomerular epithelial cells. The Journal of Clinical Investigation 1994;94(1):68–78. [PubMed: 8040293]
34. Soldi R, Mitola S, et al. Role of alphavbeta3 integrin in the activation of vascular endothelial growth factor receptor-2. The EMBO Journal 1999;18(4):882–892. [PubMed: 10022831]
35. Clemmons DR, Horvitz G, et al. Synthetic alphaVbeta3 antagonists inhibit insulin-like growth factor-I-stimulated smooth muscle cell migration and replication. Endocrinology 1999;140(10):4616–4621. [PubMed: 10499518]
36. Jones PL, Crack J, et al. Regulation of tenascin-C, a vascular smooth muscle cell survival factor that interacts with the alpha v beta 3 integrin to promote epidermal growth factor receptor phosphorylation and growth. The Journal of Cell Biology 1997;139(1):279–293. [PubMed: 9314546]
37. Maile LA, Badley-Clarke J, et al. Structural analysis of the role of the beta 3 subunit of the alpha V beta 3 integrin in IGF-I signaling. Journal of Cell Science 2001;114(Pt 7):1417–1425. [PubMed: 11257007]
38. Brooks PC, Klemke RL, et al. Insulin-like growth factor receptor cooperates with integrin alpha v beta 5 to promote tumor cell dissemination in vivo. The Journal of Clinical Investigation 1997;99 (6):1390–1398. [PubMed: 9077549]
39. Nicholson WC, Ge Z, et al. Insulin-like growth factor (IGF)-I, IGF-I receptor, and IGF binding protein-3 messenger ribonucleic acids and protein in corpora lutea from prostaglandin F(2alpha)-treated gilts. Biology of Reproduction 1999;61(6):1527–1534. [PubMed: 10569999]
40. Schneller M, Vuori K, et al. Alphavbeta3 integrin associates with activated insulin and PDGFbeta receptors and potentiates the biological activity of PDGF. The EMBO Journal 1997;16(18):5600–5607. [PubMed: 9312019]
41. Serini G, Valdembri D, et al. Integrins and angiogenesis: A sticky business. Experimental Cell Research 2006;312(5):651–658. [PubMed: 16325811]
42. Vuori K, Ruoslahti E. Association of insulin receptor substrate-1 with integrins. Science 1994;266 (5190):1576–1578. [PubMed: 7527156]
43. Doerr ME, Jones JI. The roles of integrins and extracellular matrix proteins in the insulin-like growth factor I-stimulated chemotaxis of human breast cancer cells. The Journal of Biological Chemistry 1996;271(5):2443–2447. [PubMed: 8576205]
44. Falcioni R, Antonini A, et al. Alpha 6 beta 4 and alpha 6 beta 1 integrins associate with ErbB-2 in human carcinoma cell lines. Experimental Cell Research 1997;236(1):76–85. [PubMed: 9344587]

45. Folgiero V, Avetrani P, et al. Induction of ErbB-3 expression by alpha6beta4 integrin contributes to tamoxifen resistance in ERbeta1-negative breast carcinomas. PLoS ONE 2008;3(2):e1592. [PubMed: 18270579]
46. Holmes DI, Zachary I. The vascular endothelial growth factor (VEGF) family: Angiogenic factors in health and disease. Genome Biology 2005;6(2):209. [PubMed: 15693956]
47. Lee CB, Socinski MA. Vascular endothelial growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer: A review of recent clinical trials. Reviews on Recent Clinical Trials 2007;2 (2):117–120. [PubMed: 18473996]
48. Soker S, Takashima S, et al. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 1998;92(6):735–745. [PubMed: 9529250]
49. Fong GH, Rossant J, et al. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 1995;376(6535):66–70. [PubMed: 7596436]
50. Shalaby F, Rossant J, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 1995;376(6535):62–66. [PubMed: 7596435]
51. Nishi J, Minamino T, et al. Vascular endothelial growth factor receptor-1 regulates postnatal angiogenesis through inhibition of the excessive activation of Akt. Circulation Research 2008;103 (3):261–268. [PubMed: 18583712]
52. Sato Y, Kanno S, et al. Properties of two VEGF receptors, Flt-1 and KDR, in signal transduction. Annals of the New York Academy of Sciences 2000;902:201–205. discussion 205–207. [PubMed: 10865839]
53. Petrova TV, Makinen T, et al. Signaling via vascular endothelial growth factor receptors. Experimental Cell Research 1999;253(1):117–130. [PubMed: 10579917]
54. Seetharam L, Gotoh N, et al. A unique signal transduction from FLT tyrosine kinase, a receptor for vascular endothelial growth factor VEGF. Oncogene 1995;10(1):135–147. [PubMed: 7824266]
55. Takahashi T, Yamaguchi S, et al. A single auto-phosphorylation site on KDR/Flk-1 is essential for VEGF-A-dependent activation of PLC-gamma and DNA synthesis in vascular endothelial cells. The EMBO Journal 2001;20(11):2768–2778. [PubMed: 11387210]
56. Ribatti D. The discovery of the placental growth factor and its role in angiogenesis: A historical review. Angiogenesis 2008;11(3):215–221. [PubMed: 18568405]
57. Odorisio T, Cianfarani F, et al. The placenta growth factor in skin angiogenesis. Journal of Dermatological Science 2006;41(1):11–19. [PubMed: 16216466]
58. Xu L, Cochran DM, et al. Placenta growth factor overexpression inhibits tumor growth, angiogenesis, and metastasis by depleting vascular endothelial growth factor homodimers in orthotopic mouse models. Cancer Research 2006;66(8):3971–3977. [PubMed: 16618713]
59. Byzova TV, Goldman CK, et al. A mechanism for modulation of cellular responses to VEGF: Activation of the integrins. Molecular Cell 2000;6(4):851–860. [PubMed: 11090623]
60. Mitola S, Brenchio B, et al. Type I collagen limits VEGFR-2 signaling by a SHP2 protein-tyrosine phosphatase-dependent mechanism 1. Circulation Research 2006;98(1):45–54. [PubMed: 16339483]
61. De S, Razorenova O, et al. VEGF-integrin interplay controls tumor growth and vascularization. Proceedings of the National Academy of Sciences of the United States of America 2005;102(21): 7589–7594. [PubMed: 15897451]
62. Qi JH, Claesson-Welsh L. VEGF-induced activation of phosphoinositide 3-kinase is dependent on focal adhesion kinase. Experimental Cell Research 2001;263(1):173–182. [PubMed: 11161716]
63. Trusolino L, Cavassa S, et al. HGF/scatter factor selectively promotes cell invasion by increasing integrin avidity. The FASEB Journal 2000;14(11):1629–1640. [PubMed: 10928998]
64. Inbal A, Dardik R. Role of coagulation factor XIII (FXIII) in angiogenesis and tissue repair. Pathophysiology of Haemostasis and Thrombosis 2006;35(1–2):162–165. [PubMed: 16855365]
65. Dardik R, Inbal A. Complex formation between tissue transglutaminase II (tTG) and vascular endothelial growth factor receptor 2 (VEGFR-2): Proposed mechanism for modulation of endothelial cell response to VEGF. Experimental Cell Research 2006;312(16):2973–2982. [PubMed: 16914140]
66. Wang JF, Zhang XF, et al. Stimulation of beta 1 integrin induces tyrosine phosphorylation of vascular endothelial growth factor receptor-3 and modulates cell migration. The Journal of Biological Chemistry 2001;276(45):41950–41957. [PubMed: 11553610]

67. Zhang X, Groopman JE, et al. Extracellular matrix regulates endothelial functions through interaction of VEGFR-3 and integrin alpha5beta1. Journal of Cellular Physiology 2005;202(1):205–214. [PubMed: 15389531]
68. Yang JT, Rayburn H, et al. Embryonic mesodermal defects in alpha 5 integrin-deficient mice. Development 1993;119(4):1093–1105. [PubMed: 7508365]
69. Sheppard D. In vivo functions of integrins: Lessons from null mutations in mice. Matrix Biology: Journal of the International Society for Matrix Biology 2000;19(3):203–209. [PubMed: 10936445]
70. Bader BL, Rayburn H, et al. Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. Cell 1998;95(4):507–519. [PubMed: 9827803]
71. Reynolds LE, Wyder L, et al. Enhanced pathological angiogenesis in mice lacking beta3 integrin or beta3 and beta5 integrins. Nature Medicine 2002;8(1):27–34.
72. Su G, Hodnett M, et al. Integrin alphavbeta5 regulates lung vascular permeability and pulmonary endothelial barrier function. American Journal of Respiratory Cell and Molecular Biology 2007;36 (3):377–386. [PubMed: 17079779]
73. Johnson FM, Gallick GE. SRC family nonreceptor tyrosine kinases as molecular targets for cancer therapy. Anti-cancer Agents in Medicinal Chemistry 2007;7(6):651–659. [PubMed: 18045060]
74. Kefalas P, Brown TR, et al. Signalling by the p60csrc family of protein-tyrosine kinases. International Journal of Biochemistry and Cell Biology 1995;27(6):551–563. [PubMed: 7545532]
75. Basson MD. An intracellular signal pathway that regulates cancer cell adhesion in response to extracellular forces. Cancer Research 2008;68(1):2–4. [PubMed: 18172287]
76. Koppikar P, Choi SH, et al. Combined inhibition of c-Src and epidermal growth factor receptor abrogates growth and invasion of head and neck squamous cell carcinoma. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 2008;14(13):4284–4291. [PubMed: 18594011]
77. Coluccia AM, Cirulli T, et al. Validation of PDGFRbeta and c-Src tyrosine kinases as tumor/vessel targets in patients with multiple myeloma: Preclinical efficacy of the novel, orally available inhibitor dasatinib. Blood 2008;112(4):1346–1356. [PubMed: 18524994]
78. Eliceiri BP, Paul R, et al. Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. Molecular Cell 1999;4(6):915–924. [PubMed: 10635317]
79. Schwartzberg PL, Xing L, et al. Rescue of osteoclast function by transgenic expression of kinase-deficient Src in src−/− mutant mice. Genes and Development 1997;11(21):2835–2844. [PubMed: 9353253]
80. Soriano P, Montgomery C, et al. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 1991;64(4):693–702. [PubMed: 1997203]
81. Lowell CA, Soriano P. Knockouts of Src-family kinases: Stiff bones, wimpy T cells, and bad memories. Genes and Development 1996;10(15):1845–1857. [PubMed: 8756343]
82. Klinghoffer RA, Sachsenmaier C, et al. Src family kinases are required for integrin but not PDGFR signal transduction. The EMBO Journal 1999;18(9):2459–2471. [PubMed: 10228160]
83. Salmivirta K, Talts JF, et al. Binding of mouse nidogen-2 to basement membrane components and cells and its expression in embryonic and adult tissues suggest complementary functions of the two nidogens. Experimental Cell Research 2002;279(2):188–201. [PubMed: 12243745]
84. Klemke RL, Yebra M, et al. Receptor tyrosine kinase signaling required for integrin alpha v beta 5-directed cell motility but not adhesion on vitronectin. The Journal of Cell Biology 1994;127(3):859–866. [PubMed: 7525598]
85. Eliceiri BP, Klemke R, et al. Integrin alphavbeta3 requirement for sustained mitogen-activated protein kinase activity during angiogenesis. The Journal of Cell Biology 1998;140(5):1255–1263. [PubMed: 9490736]
86. Sahni A, Francis CW. Stimulation of endothelial cell proliferation by FGF-2 in the presence of fibrinogen requires alphavbeta3. Blood 2004;104(12):3635–3641. [PubMed: 15297314]
87. Toledo MS, Suzuki E, et al. Effect of ganglioside and tetraspanins in microdomains on interaction of integrins with fibroblast growth factor receptor. The Journal of Biological Chemistry 2005;280(16): 16227–16234. [PubMed: 15710618]
88. Yancopoulos GD, Davis S, et al. Vascular-specific growth factors and blood vessel formation. Nature 2000;407(6801):242–248. [PubMed: 11001067]

89. Fiedler U, Augustin HG. Angiopoietins: A link between angiogenesis and inflammation. Trends in Immunology 2006;27(12):552–558. [PubMed: 17045842]

90. Minshall RD, Malik AB. Transport across the endothelium: Regulation of endothelial permeability. Handbook of Experimental Pharmacology 2006;176(Pt 1):107–144. [PubMed: 16999218]

91. Tsigkos S, Zhou Z, et al. Regulation of Ang2 release by PTEN/PI3-kinase/Akt in lung microvascular endothelial cells. Journal of Cellular Physiology 2006;207(2):506–511. [PubMed: 16447257]

92. Roviezzo F, Tsigkos S, et al. Angiopoietin-2 causes inflammation in vivo by promoting vascular leakage. Journal of Pharmacology and Experimental Therapeutics 2005;314(2):738–744. [PubMed: 15870388]

93. Cascone I, Napione L, et al. Stable interaction between alpha5beta1 integrin and Tie2 tyrosine kinase receptor regulates endothelial cell response to Ang-1. The Journal of Cell Biology 2005;170(6):993–1004. [PubMed: 16157706]

94. Thamilselvan V, Craig DH, et al. FAK association with multiple signal proteins mediates pressure-induced colon cancer cell adhesion via a Src-dependent PI3 K/Akt pathway. The FASEB Journal 2007;21(8):1730–1741. [PubMed: 17317726]

95. Eliceiri BP, Puente XS, et al. Src-mediated coupling of focal adhesion kinase to integrin alpha(v) beta5 in vascular endothelial growth factor signaling. The Journal of Cell Biology 2002;157(1):149–160. [PubMed: 11927607]

96. Lesko E, Majka M. The biological role of HGF-MET axis in tumor growth and development of metastasis. Frontiers in Bioscience: A Journal and Virtual Library 2008;13:1271–1280. [PubMed: 17981628]

97. Comoglio PM, Giordano S, et al. Drug development of MET inhibitors: Targeting oncogene addiction and expedience. Nature Reviews. Drug Discovery 2008;7(6):504–516. [PubMed: 18511928]

98. Bell LN, Cai L, et al. A central role for hepatocyte growth factor in adipose tissue angiogenesis. American Journal of Physiology. Endocrinology and Metabolism 2008;294(2):E336–E344. [PubMed: 18073323]

99. Rahman S, Patel Y, et al. Novel hepatocyte growth factor (HGF) binding domains on fibronectin and vitronectin coordinate a distinct and amplified Met-integrin induced signalling pathway in endothelial cells. BMC Cell Biology 2005;6(1):8. [PubMed: 15717924]

100. Chung J, Yoon SO, et al. The Met receptor and alpha 6 beta 4 integrin can function independently to promote carcinoma invasion. The Journal of Biological Chemistry 2004;279(31):32287–32293. [PubMed: 15161909]

101. Nikolopoulos SN, Blaikie P, et al. Integrin beta4 signaling promotes tumor angiogenesis. Cancer Cell 2004;6(5):471–483. [PubMed: 15542431]

102. Kawasaki T, Kitsukawa T, et al. A requirement for neuropilin-1 in embryonic vessel formation. Development 1999;126(21):4895–4902. [PubMed: 10518505]

103. Kruger RP, Aurandt J, et al. Semaphorins command cells to move. Nature Reviews. Molecular Cell Biology 2005;6(10):789–800.

104. Soker S, Miao HQ, et al. VEGF165 mediates formation of complexes containing VEGFR-2 and neuropilin-1 that enhance VEGF165-receptor binding. Journal of Cellular Biochemistry 2002;85 (2):357–368. [PubMed: 11948691]

105. Serini G, Valdembri D, et al. Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. Nature 2003;424(6947):391–397. [PubMed: 12879061]

106. Deutsch U. Semaphorins guide PerPlexeD endothelial cells. Developmental Cell 2004;7(1):1–2. [PubMed: 15239945]

107. Guttmann-Raviv N, Shraga-Heled N, et al. Semaphorin-3A and semaphorin-3F work together to repel endothelial cells and to inhibit their survival by induction of apoptosis. The Journal of Biological Chemistry 2007;282(36):26294–26305. [PubMed: 17569671]

108. Geretti E, Shimizu A, et al. Neuropilin structure governs VEGF and semaphorin binding and regulates angiogenesis. Angiogenesis 2008;11(1):31–39. [PubMed: 18283547]

109. Banu N, Teichman J, et al. Semaphorin 3C regulates endothelial cell function by increasing integrin activity. The FASEB Journal 2006;20(12):2150–2152. [PubMed: 16940438]

110. Kinbara K, Goldfinger LE, et al. Ras GTPases: Integrins' friends or foes? Nature Reviews. Molecular Cell Biology 2003;4(10):767–776.

111. Oinuma I, Ishikawa Y, et al. The Semaphorin 4D receptor Plexin-B1 is a GTPase activating protein for R-Ras. Science 2004;305(5685):862–865. [PubMed: 15297673]

112. Dallas SL, Sivakumar P, et al. Fibronectin regulates latent transforming growth factor-beta (TGF beta) by controlling matrix assembly of latent TGF beta-binding protein-1. The Journal of Biological Chemistry 2005;280(19):18871–18880. [PubMed: 15677465]

113. Vlahakis NE, Young BA, et al. Integrin alpha9-beta1 directly binds to vascular endothelial growth factor (VEGF)-A and contributes to VEGF-A-induced angiogenesis. The Journal of Biological Chemistry 2007;282(20):15187–15196. [PubMed: 17363377]

114. Vlahakis NE, Young BA, et al. The lymphangiogenic vascular endothelial growth factors VEGF-C and -D are ligands for the integrin alpha9beta1. The Journal of Biological Chemistry 2005;280(6): 4544–4552. [PubMed: 15590642]

115. Kajiya K, Hirakawa S, et al. Hepatocyte growth factor promotes lymphatic vessel formation and function. The EMBO Journal 2005;24(16):2885–2895. [PubMed: 16052207]

116. Mori S, Wu CY, et al. Direct binding of integrin alphavbeta3 to FGF1 plays a role in FGF1 signaling. The Journal of Biological Chemistry 2008;283(26):18066–18075. [PubMed: 18441324]

117. Shim WS, Ho IA, et al. Angiopoietin: A TIE(d) balance in tumor angiogenesis. Molecular Cancer Research 2007;5(7):655–665. [PubMed: 17634421]

118. Carlson TR, Feng Y, et al. Direct cell adhesion to the angiopoietins mediated by integrins. The Journal of Biological Chemistry 2001;276(28):26516–26525. [PubMed: 11346644]

119. Dallabrida SM, Ismail N, et al. Angiopoietin-1 promotes cardiac and skeletal myocyte survival through integrins. Circulation Research 2005;96(4):e8–e24. [PubMed: 15692086]

120. Hu B, Jarzynka MJ, et al. Angiopoietin 2 induces glioma cell invasion by stimulating matrix metalloprotease 2 expression through the alphavbeta1 integrin and focal adhesion kinase signaling pathway. Cancer Research 2006;66(2):775–783. [PubMed: 16424009]

121. Dallabrida SM, Ismail NS, et al. Integrin binding angiopoietin-1 monomers reduce cardiac hypertrophy. The FASEB Journal 2008;22(8):3010–3023. [PubMed: 18502941]

122. Imanishi Y, Hu B, et al. Angiopoietin-2 stimulates breast cancer metastasis through the alpha(5)beta (1) integrin-mediated pathway. Cancer Research 2007;67(9):4254–4263. [PubMed: 17483337]

123. Kalluri R. Basement membranes: Structure, assembly and role in tumour angiogenesis. Nature Reviews Cancer 2003;3(6):422–433.

124. Shiojima I, Walsh K. Role of Akt signaling in vascular homeostasis and angiogenesis. Circulation Research 2002;90(12):1243–1250. [PubMed: 12089061]

125. Wickstrom SA, Alitalo K, et al. An endostatin-derived peptide interacts with integrins and regulates actin cytoskeleton and migration of endothelial cells. The Journal of Biological Chemistry 2004;279 (19):20178–20185. [PubMed: 14973128]

126. Rehn M, Veikkola T, et al. Interaction of endostatin with integrins implicated in angiogenesis. Proceedings of the National Academy of Sciences of the United States of America 2001;98(3): 1024–1029. [PubMed: 11158588]

127. Maeshima Y, Sudhakar A, et al. Tumstatin, an endothelial cell-specific inhibitor of protein synthesis. Science 2002;295(5552):140–143. [PubMed: 11778052]

128. Sudhakar A, Sugimoto H, et al. Human tumstatin and human endostatin exhibit distinct antiangiogenic activities mediated by alpha v beta 3 and alpha 5 beta 1 integrins. Proceedings of the National Academy of Sciences of the United States of America 2003;100(8):4766–4771. [PubMed: 12682293]

129. Sudhakar A, Nyberg P, et al. Human alpha1 type IV collagen NC1 domain exhibits distinct antiangiogenic activity mediated by alpha1beta1 integrin. The Journal of Clinical Investigation 2005;115(10):2801–2810. [PubMed: 16151532]

130. Magnon C, Galaup A, et al. Canstatin acts on endothelial and tumor cells via mitochondrial damage initiated through interaction with alphavbeta3 and alphavbeta5 integrins. Cancer Research 2005;65 (10):4353–4361. [PubMed: 15899827]

131. Wahl ML, Kenan DJ, et al. Angiostatin's molecular mechanism: Aspects of specificity and regulation elucidated. Journal of Cellular Biochemistry 2005;96(2):242–261. [PubMed: 16094651]

132. Gutheil JC, Campbell TN, et al. Targeted antiangiogenic therapy for cancer using Vitaxin: A humanized monoclonal antibody to the integrin alphavbeta3. Clinical Cancer Research: An Official

Journal of the American Association for Cancer Research 2000;6(8):3056–3061. [PubMed: 10955784]

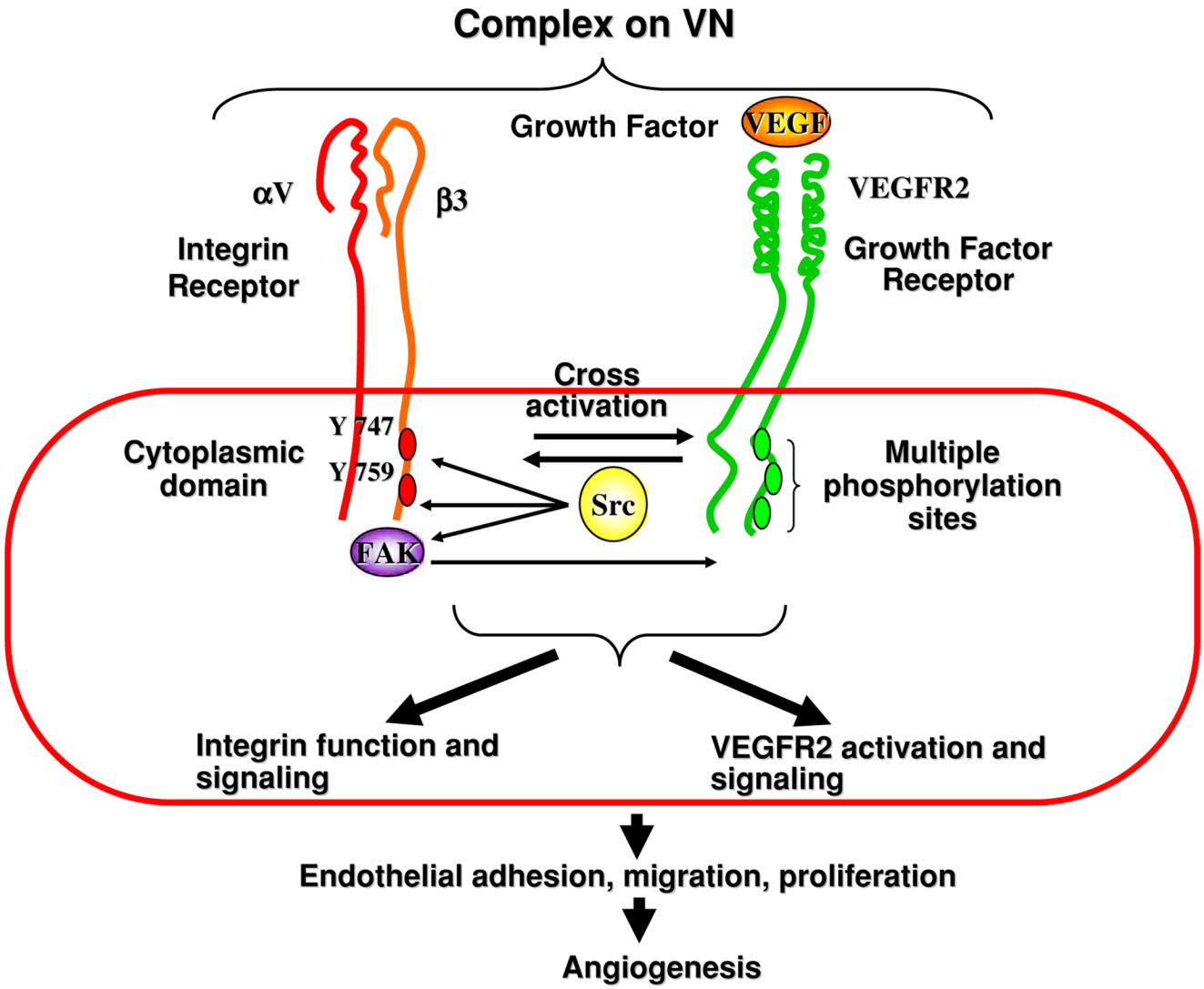

Fig. 1.

Effect of extracellular matrix proteins and VEGF on integrin $\alpha_v\beta_3$ and VEGFR-2 interaction. Engagement of endothelial β_3 integrin with extracellular matrix proteins such as vitronectin (VN) in the presence of VEGF interaction with VEGFR-2 (Flk-1) results in a number of modifications on both the receptors that trigger intracellular signaling cascade. These conformational changes and modifications by phosphorylation augment the complex formation between intgrin β_3 and VEGFR-2. Upon stimulation with VEGF, VEGFR-2 undergoes auto-phosphorylation of a number of tyrosine residues in the cytoplasmic domain. Src kinase, activated downstream of this signaling, phosphorylates two tyrosine residues in the cytoplasmic domain of β_3 integrin (Y747 and Y759) which is necessary for the interaction between the two receptors

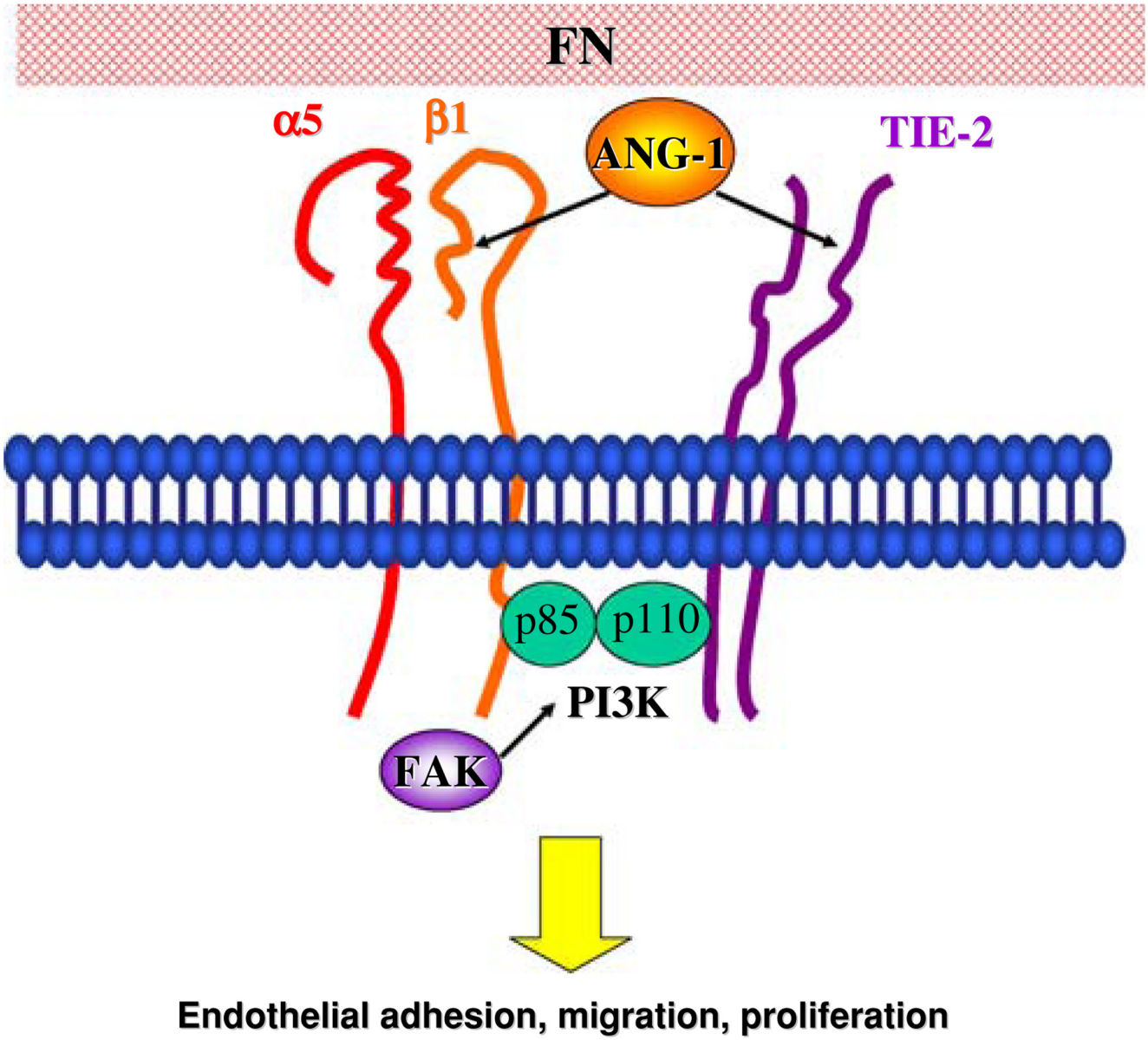

Fig. 2.

Effect of extracellular matrix proteins and Angiopoietin-1 on integrin $\alpha_5\beta_1$ and Tie-2 interaction. Activation of integrin $\alpha_5\beta_1$ by attachment to fibronectin increases its interaction with Tie-2. Upon activation of integrin $\alpha_5\beta_1$, Tie-2 is phosphorylated in the presence of the specific ligand Ang-1. In endothelial cells attached to fibronectin, Ang-1 stimulates recruitment of p85 to Tie-2 and FAK to the cytoplasmic tails of integrin $\alpha_5\beta_1$ clusters in the focal adhesions. This complex formation between Tie-2 and integrin $\alpha_5\beta_1$ involves activation of PI-3 kinase signaling. Alternatively, Ang-1 also directly binds to integrin $\alpha_5\beta_1$ even in the absence of Tie-2 receptor. Altogether, interaction between integrin $\alpha_5\beta_1$ and Tie-2 results in vascular stabilization and maturation

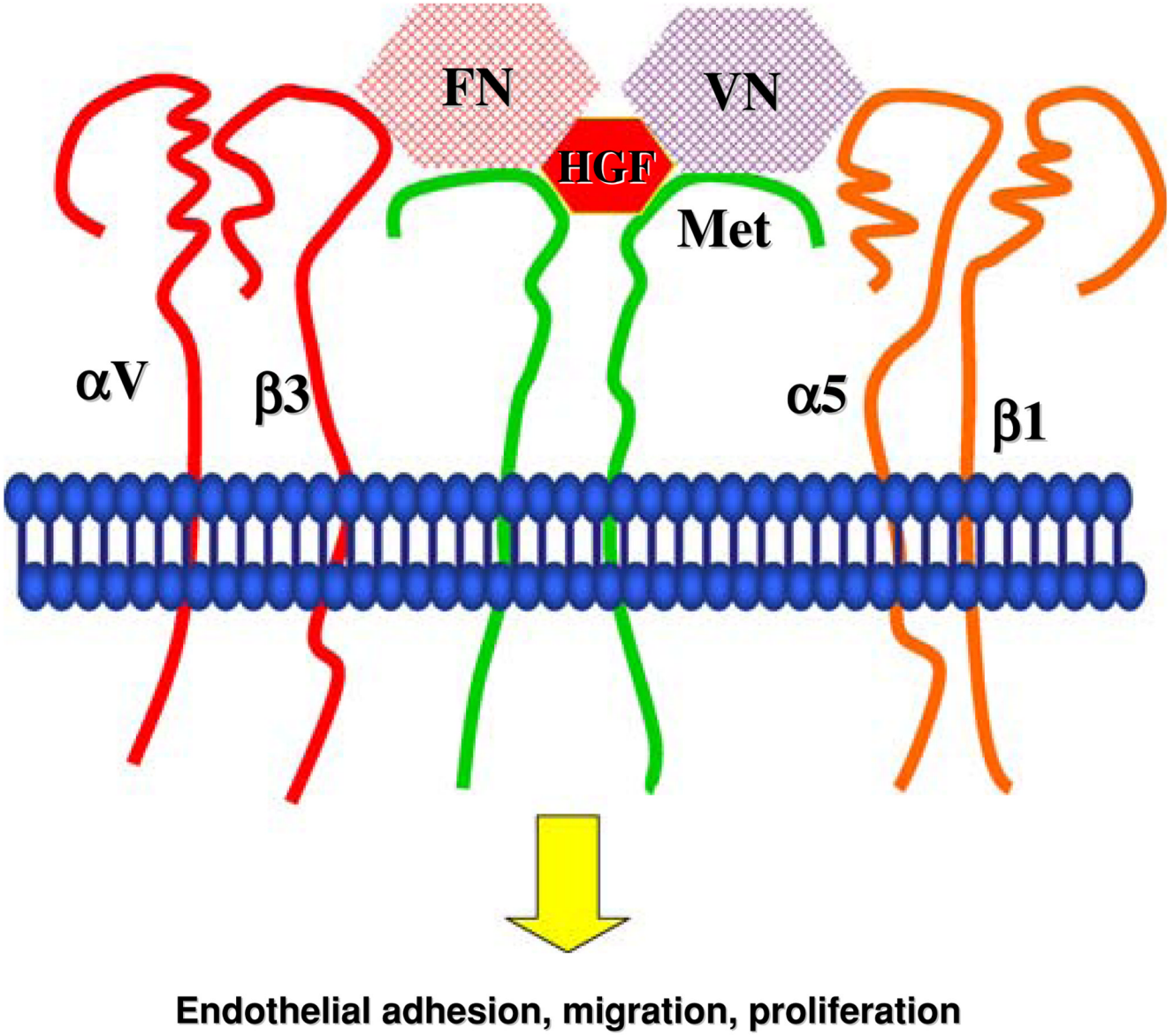

Fig. 3.

Effect of interactions between extracellular matrix proteins and hepatocyte growth factor (HGF) on complex formation among integrin $\alpha_5\beta_1$, integrin $\alpha_v\beta_3$, and Met. HGF, largely synthesized and secreted by platelets, forms hetero-complexes with vitronectin and fibronectin, two important ligands for integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$. These hetero-complexes, VN/HGF and FN/HGF, trigger association between Met and integrins $\alpha_v\beta_3$ and $\alpha_5\beta_1$, respectively. This subsequently leads to enhanced auto-phosphorylation of Met

Table 1

Direct interactions between growth factor receptors and integrins

Growth factor receptor	Integrin(s)	References
VEGFR2/*Flk1*	$\alpha_v\beta_3$	[15,34]
VEGFR3/*Flt4*	$\alpha_5\beta_1$	[66,67]
PDGFR	$\alpha_v\beta_3$	[32,40]
FGFR3	$\alpha_v\beta_3$, $\alpha_5\beta_1$	[86,87]
IRS-1	$\alpha_v\beta_3$, $\alpha_5\beta_1$	[42]
Tie-2	$\alpha_5\beta_1$	[93]
ErB-2	$\alpha_6\beta_4$, $\alpha_6\beta_1$	[43,44]
ErB-3	$\alpha_5\beta_1$	[45]
PlexinB1	$\alpha_5\beta_1$	[111]
Met	$\alpha_v\beta_3$,$\alpha_5\beta_1$,$\alpha_6\beta_4$	[99,101]

Table 2

Direct interactions between integrins and angiogenic modulators

Growth factor	Integrin(s)	References
VEGFA-165	$\alpha_v\beta_3$, $\alpha_3\beta_1$, $\alpha_9\beta_1$	[113–115]
Angiopoietin-1	$\alpha_5\beta_1$	[117]
Angiopoietin-2	$\alpha_5\beta_1$	[117]
PDGF	$\alpha_v\beta_3$	[40]
IGF	$\alpha_v\beta_5$	[38,43,84]
EGF	$\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$	[33,36,38,43,84]
Tumstatin	$\alpha_v\beta_3$	[127,128]
Arresten	$\alpha_1\beta_1$	[129]
Canstatin	$\alpha_v\beta_3$, $\alpha_v\beta_5$	[130]
Endostatin	$\alpha_5\beta_1$	[125]