Cardiovascular Research (2008) **78**, 213–222
doi:10.1093/cvr/cvn045

Review

Besides adhesion: new perspectives of integrin functions in angiogenesis

Guido Serini, Lucia Napione, Marco Arese, and Federico Bussolino*

Department of Oncological Sciences, Institute for Cancer Research and Treatment, University of Torino, Sp 142, Km 3.95, 10060 Candiolo, Torino, Italy

Received 4 January 2008; revised 5 February 2008; accepted 13 February 2008; online publish-ahead-of-print 19 February 2008

Time for primary review: 11 days

KEYWORDS
Plexin;
Semaphorin;
Tyrosine kinase receptors;
Endothelial cells

During angiogenic remodelling in embryo and adult life, endothelial cells lining blood vessel walls dynamically modify their integrin-mediated adhesive contacts with the surrounding extracellular matrix. However, besides regulating cell adhesion and migration, integrins dynamically participate in a network with soluble molecules and their receptors. Angiogenesis is characterized by opposing autocrine and paracrine loops of growth factors and semaphorins that regulate the activation of integrins on the endothelial surface through tyrosine kinase receptors (TKR) and the neuropilin/plexin system. Moreover, pro- and anti-angiogenic factors can directly bind integrins and regulate endothelial cell behaviour. This review summarizes the recent progress in understanding the reciprocal interactions between integrins, TKR, and semaphorin receptors.

1. Introduction

The molecular mechanisms leading to cell–extracellular matrix (ECM) interactions have been crucial for the evolution from protozoans to metazoans. Integrins represent the most important family of receptors mediating cell adhesion to ECM. Each integrin is composed of non-homologous transmembrane α and β subunits and they control cell adhesion through complex molecular mechanisms. Outside-in signalling informs the cell about the ECM environment, while inside-out signalling promotes modifications in integrin functional activity.[1,2]

Development and remodelling of vascular systems require complex interactions of signals and physical forces orchestrating the activities of endothelial cells (ECs), pericytes, and smooth muscle cells. Besides several redundant soluble factors, which appear to have a relevant role, two classes of molecules have been identified with a high specificity for the vascular system: the family of vascular endothelium growth factors (VEGF) and their tyrosine kinase receptors (TKR), VEGFR-1, -2, and -3, and the family of angiopoietins (Ang) and Tie-2 TKR.[3,4] More recently, molecules firstly characterized for their role in axon guidance (e.g. semaphorins, netrins, and slits) have been selectively involved in the remodelling and sprouting phases of angiogenesis.[5]

Vascular cells (i.e. ECs, pericytes, and smooth muscle cells) express a wide range of integrins including $\alpha1\beta1$, $\alpha2\beta1$, $\alpha4\beta1$, $\alpha5\beta1$, $\alpha v\beta1$, $\alpha v\beta3$, $\alpha v\beta5$, $\alpha v\beta8$, $\alpha6\beta1$, and $\alpha6\beta4$.[6] Integrin-mediated cell-to-ECM adhesion plays a deterministic role in vascular development by contributing to cell movement, to protect cells from *anoikis* and to endow the vasculature with the ability to sense and respond to changes in physical forces.[6,7]

In the last 10 years, an increasing body of evidences has demonstrated that integrins are not mere adhesion receptors, but influence the biological activity of several other molecular systems within the cell. Here, we reviewed emerging results highlighting new roles of integrins in angiogenesis.

2. Integrins modulate the activation of vascular tyrosine kinase receptors

2.1 Integrins and vascular endothelial growth factor receptors

During angiogenesis, ECs adhere to a provisional ECM mainly through $\alpha v\beta3$ integrin resulting in an increased biological response to VEGF-A dependent on the formation of a complex between the integrin and VEGFR-2.[8–11] The collagen I receptor $\alpha2\beta1$ and the laminin receptors $\alpha6\beta1$ and $\alpha6\beta4$ do not exert a similar effect. The formation of the integrin-TKR complex first requires the activation of VEGFR-2 by its ligand and the ensuing binding of phosphatidylinositol 3-kinase (PI3K)[9] and c-src,[11] which participate in directional cell migration triggered by VEGF-A. A monoclonal antibody anti-$\beta3$ not only perturbed the complex formation, but it also markedly inhibited VEGFR-2-mediated

* Corresponding author. Tel: +39 0119933347; fax: +39 09119933524.
E-mail address: federico.bussolino@unito.it

phosphorylation, PI3K activation, focal adhesion dynamics as well as EC proliferation and migration triggered by VEGF-A.[9] In contrast, αvβ3 clustering was permissive for VEGFR-2 activation and optimal response of ECs to VEGF-A.[9,10,12] Formation of the VEGFR-2/αvβ3 complex requires the extracellular domains of both αv and β3 integrin subunit[8] and that of VEGFR-2.[13] Recently, a series of elegant *in vivo* and *in vitro* studies[10,11] defined the molecular details by which the β3 cytosolic tail regulates the endothelial response to VEGF-A. Indeed, upon VEGF-A stimulation, VEGFR-2 recruits and activates c-src, which in turn phosphorylates the cytosolic tail of β3 integrin at Tyr747 and Tyr759. This c-src-dependent post-translational modification is required for the formation of the VEGFR-2/αvβ3 complex and the conformational activation of the integrin, which enhances its affinity for the ECM[11] (*Figure 1*). Recently, coagulation factor FXIII has been reported to play a key role in the stabilization and activation of the VEGFR-2/αvβ3 complex. In such a complex, VEGFR-2 is activated in a VEGF-A-independent manner that requires both the transglutaminase and tyrosine kinase enzymatic activities of FXIII and VEGFR-2, respectively.[14]

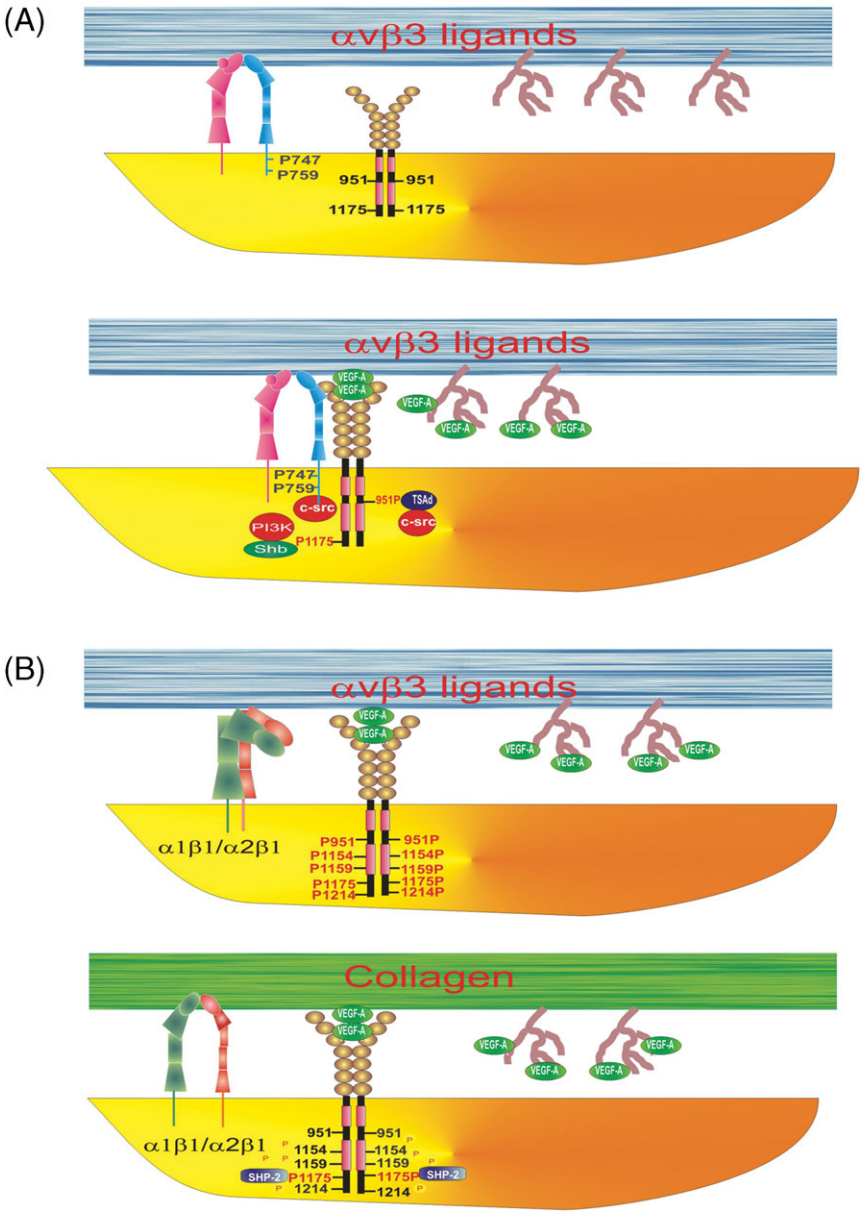

Figure 1 Effect of different ECM proteins and integrins on VEGFR-2 response. (*A*) Effect of αvβ3/VN pair on VEGFR-2 activation. VEGFR-2 triggering by VEGF-A activates a complex of signals involving a direct interaction of c-src to β3 integrin through the sequence YRGT762 [114] and to VEGFR-2 through the adaptor T--cell-specific adaptor (TSAd).[4] The figure points out the effect of longer VEGF-A isoforms (145, 165, and 189), which bind heparansulphates through a basic sequence; however, the same activity is shared by shortest VEGF-A121. This results in: (i) an association between the β3 integrin and VEGFR-2, which depends on VEGFR-2 extracellular domain, αv extracellular domain and the β3 subunit; (ii) an increased recruitment of PI3K, (iii) an increased level of VEGFR-2 phoshorylation, (iv) an increased activity of C-Src associated to β3 integrin, and (v) an enhancement of the biological properties of VEGF-A on EC. (*B*) Effect of α1β1 or α2β1/collagen I pair on VEGFR-2 activation. The engagement of integrins α1β1 or α2β1 by collagen I promotes the association of SHP-2 to Y1174 of VEGFR-2 promoting a fast dephosporylation of the receptor, and enhancement of its internalization and a reduction of receptor responsiveness to VEGF-A. It is possible that SHP-2 is recruited from the cytosolic tail of the integrin to the receptor (see text for details).

All the information obtained from experiments on cultured ECs require to be compared with data resulting from the analysis of β3 null mice that are alive without gross anatomical defects (reviewed by Hynes[6]), with the only exception of coronary defects.[15] Furthermore, β3 null mice show an increased tumour-associated VEGF-A-dependent angiogenesis, which depends on VEGFR-2 over-expression (reviewed by Hynes[6]). Hence, in this genetic model, it seems that αvβ3 acts as a negative regulator of the VEGF-A/VEGF-R2 pathway, a role that is at odds with the anti-angiogenic effects obtained by αvβ3 antagonists. In this case, it has been proposed that antibodies and drugs interacting with αvβ3 could act as agonist of negative signals.[6] Alternatively, the increased VEGFR-2 expression observed in β3 null mice could represent a molecular compensation that further put emphasis on the importance of VEGFR-2/αvβ3 integrin co-regulation in ECs. The latter hypothesis is supported by the recent finding that in knock-in mice expressing a β3 mutant unable to be phosphorylated in Tyr residues and to form a complex with VEGFR-2, tumour angiogenesis is impaired.[11] Indeed, β3 integrin could play different or even opposite roles depending on different critical factors such as: (i) the phases/types of angiogenesis (e.g. sprouting, intussusception, or fusion); (ii) the ECM ligands and fragments (e.g. tumstatin or canstatin) engaged; (iii) the association/crosstalk with other receptors or extracellular proteins, such as FXIII[14] or milk fat globule/EGF factor 8.[16]

In degranulating platelets, VEGF-A has been reported to interact with the C-terminal heparin-II domain of fibronectin (FN) and enhance its motility activity on EC.[17,18] This effect results from an association between VEGFR-2 and α5β1, which is exclusively dependent on immobilized VEGF-A/fibronectin (FN) complex.

In contrast to the αvβ3/vitronectin (VN) pair, collagen I, the ligand of α1β1 and α2β1 integrins, exerts an inhibitory action on this TKR.[19] EC adhesion to collagen I reduces VEGF-A-induced VEGFR-2 autophosphorylation by recruiting the tyrosine phosphatase SHP2 to the phosphorylated Tyr1117 of the receptor cytosolic tail. The interaction of SHP2 with VEGFR-2 is strictly dependent on EC adhesion to collagen I. The highest VEGFR-2 de-phosphorylation correlates with the highest degree of its internalization. We speculate that the pro-endocytic and inhibiting activity exerted by SHP2 on VEGFR-2 could be crucial to allow an accurate response of ECs migrating along VEGF-A gradients, as revealed by studies in *Drosophila melanogaster*.[20] The effect of collagen I on VEGFR-2 parallels the effect of tissue inhibitor of metalloprotease (TIMP) -2, which negatively regulates VEGFR-2 by activating SHP1 phosphatase.[21] TIMP-2/$\alpha_3\beta_1$ integrin signalling, via SHP-1 activation, inhibits cell-cycle and enhances the expression of the anti-migratory membrane protease inhibitor RECK (reversion-inducing-cysteine-rich protein with kazal motifs), finally resulting in angiogenesis inhibition.[22,23] Upon ECs stimulation with TIMP-2, SHP1 shifts from $\alpha_3\beta_1$ integrin to VEGFR-2, which is de-phosphorylated and unable to trigger proliferation. Similarly, $\alpha_1\beta_1$ integrin engaged by collagen I activates the T-cell protein tyrosine phosphatase function that inhibits EGF receptor signalling.[24,25] It has been reported that in vascular smooth muscle cells αvβ3 engagement by VN results in tyrosine phosphorylation of β3 cytosolic domain and recruitment of SHP2, which modulate the activity of insulin growth factor I receptor.[26] Thus, we hypothesize a protective role on VEGFR-2 signalling by VN-engaged αvβ3, which recruits SHP2 and preserves the receptor from phosphatase activity. In contrast EC adhesion on collagen I, which is mediated by α1β1 but not by αvβ3, could allow SHP2 interaction with VEGFR-2 (*Figure 1*).

The modulatory role of integrins on VEGFRs is not restricted to the type 2. Stimulation of VEGFR-3 by VEGF-C or VEGF-D plays a major role in lymphangiogenesis. It has been demonstrated that VEGF-C induces VEGFR-3 to associate with α5β1. Furthermore, integrin α5β1 ligation by FN is required for the optimal activation of VEGFR-3 signalling, not only at the receptor level, but also at its downstream PI3K/Akt pathway.[27] Similarly, it has been demonstrated that β1 integrin engaged by FN or collagen transactivates VEGFR-3 by promoting the physical association between the integrin and the TKR.[28]

2.2 α5β1 integrin and the angiopoietin receptor Tie2

The Ang family and its Tie-2 TKR are required for correct organization and maturation of the newly formed vessels. *In vitro*, Tie-2 activation elicits EC adhesion, motility, and survival, while Ang-2 has an inhibitory effect.[3] We have recently reported in ECs that Tie2 may be present both in free-form and associated with α5β1 integrin.[29] The activation of α5β1 integrin by FN increases its interaction with Tie2 and modulates the time and concentration window of the receptor activation. When α5β1 is activated, Tie2 is phosphorylated at lower Ang-1 concentrations than those required on other ECM proteins. Furthermore, α5β1/Tie-2 complex allows the prolonged stimulation of Tie2 tyrosine kinase activity up to 1 hour, while free Tie-2 activation is shorter and more transient. Therefore, it seems that α5β1 activation could influence Tie2 signal duration and strength. Ang-1 triggers biochemical signals that recruit to the complex the p85 regulatory subunit of PI3K and focal adhesion kinase (FAK). It is known that p85 binds activated Tie2,[3] whereas FAK is recruited to the cytosolic tail of clusterized integrins at focal adhesions.[7] Thus, Ang-1 stimulation triggers both Tie2 and α5β1 signalling, and allows a cross-talk between these pathways by modulating Tie2/α5β1 complex. Our observation that Ang-1/Tie2 promotes the α5β1-dependent activation of PI3K signalling, which is known to depend on FAK,[30] suggests that FAK recruitment to Tie2/α5β1 complex could be dependent on activated Tie2 inside-out signalling (*Figure 2*).

2.3 Integrins and the hepatocyte growth factor receptor Met

Besides these relatively specific angiogenic regulators, vascularization is under the control of pleiotropic molecules. One of them is hepatocyte growth factor (HGF), which activates its TKR Met on EC[31] and promotes angiogenesis in a large variety of models. Moreover, HGF has been found to promote integrin-mediated adhesion.[32] When HGF is released by platelets forms a complex with FN or VN. These hetero-complexes, but not HGF alone, trigger the association between Met and integrins. In particular HGF/FN and HGF/VN, respectively, induce Met to associate

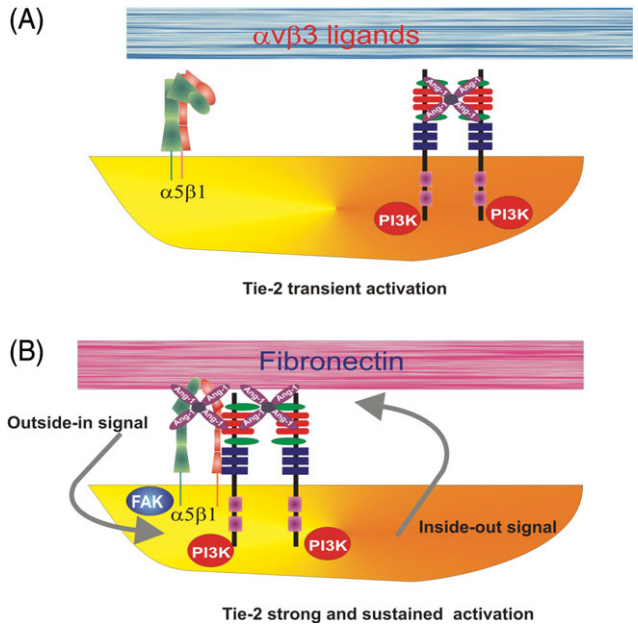

Figure 2 Effect of α5β1/FN pair on Tie-2 response to Ang-1. (*A*) Free-Tie-2 responds to high Ang-1 concentrations with transient activation. (*B*) Integrin α5β1 activated by fibronectin forms a complex with Tie-2, which in turn responds to a low Ang-1 concentration for long-lasting time; Tie-2 stimulation supports an integrin inside-out signal. The signal coming from α5β1/Tie-2 complex is further reinforced by a direct α5ß1 integrin stimulation by Ang-1. The stimulation results in a combined activity of inside-out and outside-in signals.

with α5β1 and αvβ3 integrins, with subsequent sustained level of auto-phosphorylation when compared with the non-associated receptor.[33] Since it has been found that in cancer cells Met can complex with α6β4 integrin,[34,35] known to be a regulator of new blood vessel formation in cancers,[36] it is tempting to speculate about a possible regulatory role of HGF/Met activity via α6β4 during tumour angiogenesis.

2.4 Integrins and the fibroblast growth factor receptors

By using specific neutralizing antibody anti-αvβ3, Cheresh's group suggested that this integrin cooperates with the TKRs of fibroblast growth factor (FGF)-2 to promote signalling events necessary for vascular survival and endothelial cell motility, thereby facilitating angiogenesis.[37] Specific peptide antagonists of αvβ3 integrin inhibit the second and late wave of FGF-2-mediated activation of mitogen-activated protein kinase (MAPK) resulting in an inhibition of angiogenesis.[38] Further studies allowed a better definition of the signals upstream MAPK and regulated by αvβ3 integrin. The sustained activation of MAPK by FGF-2/αvβ3 depends on p21-activated kinase, which phosphorylates c-Raf.[39] However, the molecular mechanisms of the described cooperation between FGF-2 and αvβ3 integrin are far to be elucidated. A first hint is the demonstration in ECs that activated FGR receptor-1 binds this integrin engaged by fibrionogen.[40] This result parallels other data showing an association between FGF receptor-3 and a wide number of integrins.[41]

3. Integrins bind angiogenic modulators

3.1 Integrins bind vascular endothelial growth factors

ECM can display a significant avidity for soluble molecules with the consequent variation of their diffusivity and affinity for their cognate receptors. Emerging evidences demonstrate that both angiogenic inducers and inhibitors may be entrapped by ECM and engage with integrins. Actually, α9β1 integrin forms a complex with both immobilized VEFG-A_{165} and VEGF-A_{121}.[42] EC adhere to and migrate on both isoforms using α9ß1. In response to immobilized VEGF-A, VEGF-R2 and α9ß1 assemble together and signal in an additive manner through phosphorylation of the downstream intermediates ERK and paxillin. Importantly, this complex seems to be operative *in vivo* because an antibody anti-α9β1 integrin partially reduces the angiogenic effect of VEGF-A.[42] The same integrin has been reported to interact with VEGF-C, VEGF-D, and HGF, a finding that may help explain the abnormal lymphatic phenotype of mice expressing a null mutation of the α9 subunit.[43,44] Similar observations have been reported for α3β1 and αvβ3;[45] these integrins bind to VEFG-A_{165} and VEGF-A_{189}, but not to the shorter isoform VEGF-A_{121} allowing EC migration and survival in a VEGF receptor-independent way (*Figure 3*). It is possible that the integrin/VEGF interaction could account at least in part for the diverse biological activities of VEGF-A isoforms and their ability to differently interact with ECM and co-receptors.[4,46,47] Genetically modified mice show that the different VEGF-A isoforms can influence vascular patterning.[46,47] These differences in the activity of VEGF-A isoforms could be likely due to their different ability to bind and interact with components of ECM.

3.2 Integrins bind angiopoietins

Integrins are non-endothelial-specific receptors for Ang and partially mediate the biological activities of this protein family. The molecular determinants of Ang involved in integrin binding are presumably localized in the fibrinogen-like domain; here it localizes the sequence QHREDGS, which resembles the integrin motifs KRLDGS or REDV of fibrinogen and FN, respectively.[29,48–50] EC, as well as fibroblasts,

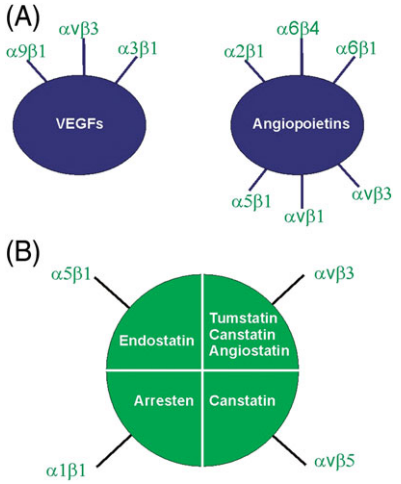

Figure 3 Interactions between inducers (*A*) and inhibitors (*B*) of angiogenesis and integrins (see text for details).

adhere to Ang-1 and -2, but only the former is able to induce cell spreading, haptotaxis, and a complete activation of cytoskeleton dynamics. β1 integrin heterodimers, and in particular α5β1, seem to be the most efficient adhesive receptors for Ang-1 independently from the presence of Tie-2.[29,50] This observation, together with the observed cooperation between Tie-2 and α5β1 (see above), indicates that Ang-1 is capable of triggering both inside-out and outside-in signals. Indeed different events may occur in vascular ECs: (i) when high amounts of Ang-1 are available both high affinity Tie-2 receptor and low affinity/high avidity integrins may be independently activated; (ii) low Ang-1 concentrations, which are unable to sustain prolonged Tie-2 and integrin activation, can instead efficiently signal only through the fraction of Tie-2 that is constitutively associated with α5ß1 and make ECs more sensitive to low amounts of Ang1.

In other cell types lacking Tie-2, Ang interact only with integrins. In cardiomyocytes, a wider range of integrins (α2β1, α5β1, α6β1, α6β4, αvβ1, and αvβ3) bind Ang-1 and Ang-2, resulting in cell survival due to Akt activation and caspase-3 inhibition.[48] Glioma cells adhere to Ang-2 via α3, α5, αv, β1, and β3, but only αvβ1 engagement by Ang-2 sustains efficient signalling that results in matrix metalloprotease-2 production, cell migration, and invasion.[49] On breast cancer cells instead adhere and migrate towards Ang-2, by using α5β1.[51] Collectively these data suggest that each cell type binds Ang through a distinct set of integrins, which only partially overlaps among different cell types (*Figure 3*).

3.3 Integrins bind angiogenesis inhibitors

A significant number of angiogenesis inhibitors derive from the proteolytic cleavage of ECM proteins[52] and therefore it is not surprising that they bind integrins (*Figure 3*). The interaction of integrins with their canonical and intact ECM ligands results in EC adhesion and activation of anti-apoptotic signals.[7] On the contrary, integrins engaged by these ECM-protein fragments promote apoptosis and reduce proliferation and motility. Altogether these data suggest that different and opposing outside-in signals are triggered by integrins depending on whether they are engaged by either intact ECM protein or fragments deriving from its cleavage.

Endostatin, the C-terminal non-collagenous domain of type XVIII collagen, exerts its inhibitory activity on EC migration mainly by interacting with α5β1. An Arg-rich peptide at the N-terminus of endostatin seems to be important for its interaction with β1,[53] supporting previous data excluding the role of an Arg-Gly-Asp sequence.[54] However, it has been also reported that Arg-Gly-Asp cyclic peptides inhibit EC binding to immobilized endostatin,[55] implying that soluble or immobilized endostatin differently interacts with α5β1. It is conceivable that α5β1 conformation is different when it binds an intact ECM ligand or endostatin, thus leading in the latter case to aberrant and perturbed signalling events, such as sustained activation of src, inhibition of FAK and MAPK.[55,56]

The non-collagenous domain present in the α chains of type IV collagen generates three different angiogenic inhibitors: tumstatin, arresten, and canstatin. Tumstatin is the non-collagenous domain of the α3 chain of type IV collagen that induces apoptosis and inhibits EC proliferation through its binding to αvβ3 integrin, leading to suppression of cap-dependent protein translation. The tumstatin/αvβ3 interaction is independent from the Arg-Gly-Asp binding site and therefore it may explain the inhibitory signals triggered by this interaction. Actually tumstatin inhibits the activation of FAK, Akt, and mTOR (target of rapamycin) -mediated phosphorylation of the eukaryotic initiation factor 4E-binding protein involved in the control of protein synthesis.[55,57] Arresten corresponds to the non-collagenous domain of the α1 chain of type IV collagen and blocks EC functions by competing with collagen IV binding to α1ß1 integrin and inhibiting MAPK-mediated signals.[58] Finally, canstatin corresponds to the α2 chain of type IV collagen, binds αvβ3 and αvβ5 leading to activation in ECs of an apoptotic program involving both caspase-8 and -9.[59] The activation of both caspase-8 and -9 results in the amplification of mitochondrial-dependent apoptotic events and in the activation of caspase-3, the central executioner of the apoptotic process. A similar process is activated by angiostatin, which corresponds to the N-terminal four kringles of plasminogen and by binding αvβ3 triggers the non-mitochondrial caspase-8-dependent apoptotic pathway in ECs.[59,60]

4. Semaphorins regulate endothelial integrin function and angiogenic remodelling

Semaphorins (Sema) are a family of secreted and membrane-bound repulsive cues, which have been originally identified for their ability to affect axon behaviour in the developing nervous system.[61] Semaphorins signal through four classes of plexins, named type A-D, a family of membrane receptors characterized by the presence in their cytosolic tail of 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.[61] In vertebrates, members of the secreted class 3 Sema employ Neuropilin (Nrp)-1 or -2 as co-receptors in association with type A or type D plexins.[61]

4.1 Semaphorins and vascular development

The first evidences for a role of Sema/Nrp/plexin system in vascular biology were provided by the groups Klagsbrun and Fujisawa, which respectively demonstrated that in ECs Nrp-1 acts as VEGFR-2 co-receptor[62] and found that Nrp-1 is required for mouse cardiovascular development.[63] Afterwards, several reports confirmed and extended these observations. In zebrafish, knockdown of *sema3aa* affects the migration of Nrp-1^{+} angioblasts, finally impairing dorsal aorta formation and normal circulation.[64] Additionally, single morpholino knockdown of either *sema3aa* or *sema3ab* in *TG(fli1:EGFP)^{y1}* embryos results in a less dramatic phenotype with patterning defects of intersomitic vessels.[65] Knockdown of *Sema3a* gene in outbred CD-1 mouse strain and over-expression of dominant negative Sema3 receptor mutants[66] or delivery of anti-Sema3A antibodies[67] in chick embryos were found to cause angiogenic remodelling defects. In a different colony of outbred CD-1 mice, *Sema3a* knock down did not result in vascular defects.[68] These discrepancies could be due to differences in the genetic background. Indeed, outbred stocks undergo

genetic heterogeneity depending on colony maintenance; moreover, even within the same outbred colony the genetic background can change over time.[69]

The observation that both *in vitro* and *in vivo*[66] angiogenic ECs display autocrine loops of several Sema3 other than Sema3A[70–73] together with the partial penetrance of the vascular phenotype in *Sema3a*$^{-/-}$ mice suggest that multiple Sema3 could cooperate to regulate angiogenesis. Notably, during angiogenesis and in cultured ECs, opposing autocrine loops of Sema3A[66,70,71,73] and VEGF-A[72,74–77] have been found and the observed loss of autocrine Sema3A in favour of VEGF-A in ECs during malignant tumour progression[73] could account at least in part for the structural and functional abnormalities of tumour vasculature.

In ECs plexinD1[78] and, albeit to lesser extent, plexinA2[79] are the most abundant plexins. Both Sema3A and Sema3C bind with a significantly higher affinity to a receptor complex formed by the association of Nrp-1 and/or -2 with plexinD1 than to a complex in which Nrps associates with plexinA1.[78] Therefore, the Nrp/plexinD1 complex could represent the most efficient transducer of the chemorepulsive effect of Sema3A.[72,73,80–82] Different from other Sema3, Sema3E can directly bind to plexinD1.[83] Mainly based on defects in the intersomitic vessel patterning of Sema3E and plexinD1, Sema3E/plexinD1 has been proposed to be the major signalling pathway regulating vascular development.[83] However, while *Sema3e* null mice are viable and do not show any gross abnormality,[83] all *plexinD1*$^{-/-}$ pups become cyanotic shortly after birth and succumb within 24 h because of severe cardiovascular defects.[78] Therefore, it is likely that in ECs, plexinD1 transduces signals not only from Sema3E, but also from other Sema3 likely employing Nrp as co-receptors, as originally proposed by Gitler and colleagues.[78] In this respect, it is worth noting that in neurons the simultaneous presence of Nrp1 and plexinD1 on the cell surface converts Sema3E/plexinD1 signalling from repulsive to attractive.[84] Based on the fact that ECs express high levels of both Nrp1 and plexinD1 and on observations that Sema3E promotes tumour angiogenesis,[85,86] at present these findings cannot be easily reconciled with the proposed chemorepulsive effect played by Sema3E via plexinD1 on ECs of developing mouse embryos.[83] Finally, the recent observation that Sema4A upon binding to plexinD1 inhibits EC migration and *in vivo* angiogenesis further indicates that plexinD1 conveys to ECs signals from multiple Sema.[87]

The complexity of Sema system in vasculature is further supported by the recent data showing that Sema3A is a powerful vasopermeabilizing molecule through a mechanism that is independent from its effects on integrin functions and involves VE-cadherin phosphorylation.[88]

4.2 Semaphorins regulate integrin function

By investigating the cellular and molecular events by which Sema can regulate vascular development, we found that Sema3A impairs EC adhesion and migration by inhibiting integrin activation.[66] Accordingly, after few minutes of stimulation with Sema3A, adherent ECs lose their focal adhesions and then collapse,[82] and Sema3F causes EC retraction as well.[89] Moreover, Sema3A and Sema3F have been shown to inhibit integrin activation and adhesion to the ECM in several cell types.[90–94] Interestingly, Sema3C, which contains an Arg-Gly-Asp motif, has been instead reported to promote EC adhesion and migration.[95]

It is well known that integrins undergo conformational modifications that regulate their affinity for ECM ligands.[2] In the low affinity state, the integrin extracellular domain is bent over the plasmamembrane and the α and β cytoplasmic tails tightly interact. In ECs chemokines, growth factors, or fluid shear stress activate signalling pathways that finally favour the transition of integrins towards the high affinity state.[2,7] Direct interaction of talin trefoil FERM (Protein 4.1, the ezrin, radixin, moesin) domain with the cytodomain of integrin β subunits results in the unclasping of the cytoplasmic tails of integrin α and β subunits together with the extension of the extracellular domain that, by exposing the ECM binding site, accomplish the transition of integrins to the high affinity state.[96] In talin, the integrin binding site within the trefoil FERM domain is masked by an intramolecular interaction with the rod domain. Interaction of membrane phosphatidylinositol 4, 5 bisphosphate (PIP2) with talin rod domain lessen this inhibition, thus allowing talin head-to-tail dimerization and integrin binding to the FERM domain. Notably, once activated talin can in turn bind and activate the enzyme phosphatidylinositol-4-phosphate 5-kinase (PIPKIγ 661) that, producing further PIP2, gives rise to a positive feed-back loop with talin that stabilizes cell adhesion to the ECM.[97] In addition, integrin function is also regulated by Rap 1 and R-Ras small-GTPases.[98] Specifically, activated R-Ras-GTP localizes at adhesive sites through its C-terminal tail;[99] here R-Ras is thought to promote cell adhesion by favouring the activation of other small GTPases, such as Rap1 and Rac1.[100] In this regard, it has been recently shown that binding of activated R-Ras to RLIP (Ral interacting protein) 76 leads to Arf (ADP-ribosylation factor) 6 activation, which promotes adhesion-induced GTP loading of Rac1.[101] In the presence of Nrp-1, the juxtamembrane basic sequence of class A plexins directly binds to FARP2, a Rac (ras-related C3 botulinum toxin substrate 1) guanosine exchange factor (GEF).[94] Sema3A binding to the Nrp-1/plexinA1 receptor complex triggers the dissociation of FARP2 from PlexinA1. Afterwards, FARP2 (FERM, RhoGEF, and pleckstrin domain protein 2) is free to exert its GEF activity leading to a rapid increase of active Rac1-GTP that in turn favours the binding of the constitutively active small GTPase Rnd1 (Rho family GTPase 1) to the linker region of plexinA1 cytoplasmic (*Figure 4*). This event finally activates PlexinA1 latent R-Ras GAP activity that then switches-off R-Ras, thus inhibiting integrin function. Importantly, Toyofuku and colleagues also found that, similarly to talin, FARP2 contains FERM domain by means which it binds plexinA1. Once detached from plexinA1, the FERM domain of FARP2 competes with talin for binding to PIPKIγ661, finally impairing the PIP2-based positive feedback required for the formation of focal adhesions.

EC migration and angiogenesis are also regulated by transmembrane Sema4D, which, depending on the cellular context, can however exert either chemoattractive or chemorepulsive activities. Negishi's laboratory provided the first evidence that plexins are endowed with an R-Ras GAP enzymatic activity by showing that Sema4D-mediated plexinB1 stimulation suppresses R-Ras activation[102] that

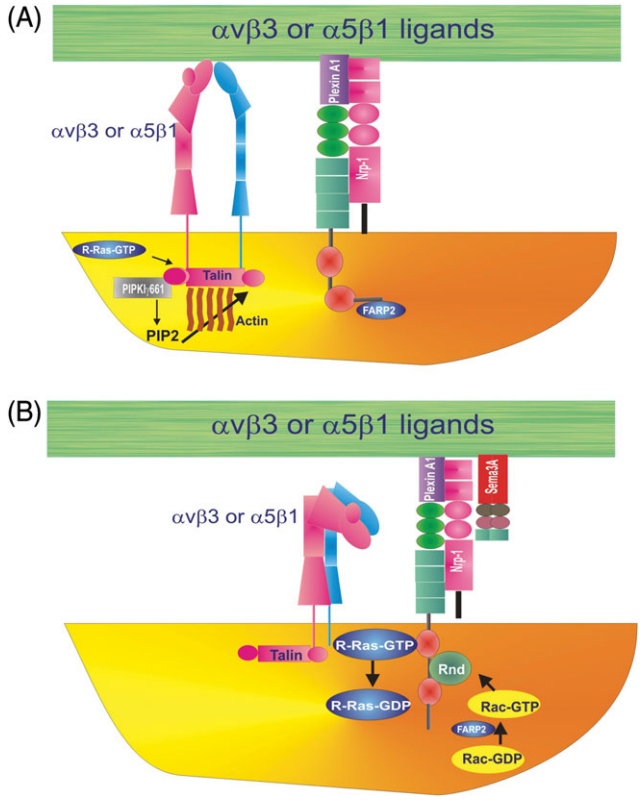

Figure 4 Effect of Sema3A on the inhibition of integrin function. (*A*) On the cell surface, αβ integrin heterodimers exist in a high affinity state, stabilized by the interaction of talin head (FERM domain) with the β subunit tail. The interaction between talin and the actin cytoskeleton occurs. Talin-activated PIPKIγ661 generates a PIP2-based positive feedback loop, which amplifies talin activation. The small GTPase R-Ras participates in integrin activation through a still poorly characterized mechanism. In the absence of SEMA3A, plexinA1 associates with Nrp-1 and the FERM domain-containing guanosine exchange factor FARP2. (*B*) Upon SEMA3A binding, FARP2 is released from plexinA1. Free FARP2 then activates Rac and favours Rnd-1 association with and activation of plexinA1 cytoplasmic GAP domain, which in turn inhibits R-Ras and integrin function. Moreover, released FARP2 inhibits PIPKIγ661 activity.

in turn inhibits β1 integrin activation.[103] Sema4D-driven R-Ras inhibition depends on the RasGAP activity triggered by Rnd1 binding to plexinB1.[102,103] However, the inhibitory activity of Sema4D is not a general phenomenon. Indeed, as first reported by Gutkind and colleagues[104] and then by us,[105] Sema4D is pro-angiogenic and elicits EC migration.[106] Sema4D stimulatory activity on ECs requires the formation of a complex between plexinB1 and Met TKR.[105] In addition, upon treatment with Sema4D plexinB1 receptor associates with a Rho-GEF capable of activating Rho GTPase and its downstream effector Rho kinase, which by phosphorylating myosin light chain could control the assembly and the contraction of actin stress fibres.[106] Altogether these data indicate that Sema4D/plexin B1 mediate different and sometimes opposite effects depending on the cellular context. As recently suggested, this may be caused by plexinB1 association with different TKR receptors; indeed, in carcinoma cells Sema4D can have pro- and anti-migratory effects depending on the interaction, respectively, with either Met or ErbB-2 TKRs.[107]

4.3 Is FAK a converging node for signals triggered by VEGF-A, integrins, and semaphorins?

Engagement of integrin receptors with their extracellular ligands leads to the formation of well-defined structures, termed focal adhesions, linking the ECM, and cytoplasmic actin cytoskeleton. These adhesive structures, which are dynamic and composed by a wide array of transmembrane and cytosolic proteins, serve as sites of force transmission required for cellular movements. Indeed, the coordinated regulation of formation and turnover of focal adhesions is central to cell responses to chemotactic and chemokinetic stimuli.[108] A primary element of focal adhesion is FAK, a kinase that is primarily recruited to sites of integrin clustering via interactions between its C-terminal region and integrin-associated proteins such as talin and paxillin. The cytosolic tail of β integrins facilitates FAK activation probably involving FAK clustering and autophosphorylation of Tyr 379 (reviewed by Mitra and Schlaepfer[30]). Furthermore, FAK is a downstream effector of several TKRs, including VEGFR-2 and FGF receptors in ECs.[39,109,110] VEGF-A via Src induces the site-specific tyrosine phosphorylation of FAK on Tyr 861, leading to the formation of a complex between FAK and αvß5, which is essential for the vascular permeability induced by VEGF-A.[111]

Recent evidences indicate that the repulsive or attractive functions of semamaphorins can involve FAK as well. Indeed, Sema3B can attract neurons by inducing membrane re-localization of phosphorylated FAK, which in turns activates the cytosolic tyrosine kinase Fyn.[112] Similarly, Sema4D elicits EC motility and angiogenesis[104,105] by activating proline-rich tyrosine kinase-2, a non-receptor tyrosine kinase closely related to FAK.[106] In contrast, both in cultured ECs and in chick chorionallantoic membrane, chemorepulsive Sema3A reduces the basal phosphorylation of Src and FAK, and induces a rapid disappearance of focal contacts followed by the collapse of the actin cytoskeleton,[82,88] thus supporting its role in inhibiting integrin function.[66]

In addition to FAK and integrins, many other proteins localize to focal adhesion, including VEGFR-2.[113] Even though the membrane topology of semaphorin receptors is still unknown, it is tempting to speculate that ECM adhesive structures could represent '*rendez-vous*' points, where multiple cross-talks between positive and negative regulators of integrin function take place.

5. Conclusions

Angiogenesis is a relevant target for the treatment of many diseases. Inhibition of angiogenesis is the aim of protocols developed for tumours, chronic inflammatory diseases, and retinopathies, while vascular regeneration inspires therapeutic angiogenesis in the treatment of ischaemic injuries. Within the molecules investigated as exploitable targets for angiogenic therapies, VEGF-A and its receptors are considered the most promising. However, there are tremendous differences between pre-clinical and clinical results. The data reviewed here clearly indicate that the activity of every drug has to be considered in connection with the robust network of regulatory signals that controls angiogenesis. Indeed, the vast accumulation of experimental and clinical reports on tumour angiogenesis can be revisited in light of the mechanisms by which cancer modifies

the robustness of regulatory networks leading to the observed abnormal vascularization, the final aim being to pharmacologically restore the robustness of the angiogenic regulatory networks in the everyday clinical practice. This interpretation might explain why some tumours display lack of sensitivity and others develop resistance to anti-VEGF-A therapy.

Funding

This work has been partially supported by Telethon (grant: GGP04127), Associazione italiana per la ricerca sul cancro (AIRC), European Community (LSHM-CT-2003-503254), Regione Piemonte (Ricerca Finalizzata 2006, Ricerca Scientifica applicata 2004 grants D10 and A150, Ricerca industriale e competitiva 2006: grant PRESTO), Fondazione Cassa di Risparmio di Torino, Torino, and 'Ministero della Salute' (Programma Ricerca Oncologica 2006, Ricerca Finalizzata 2006).

Conflict of interest: none declared.

References

1. Moissoglu K, Schwartz MA. Integrin signalling in directed cell migration. *Biol Cell* 2006;**98**:547–555.
2. Luo BH, Carman CV, Springer TA. Structural basis of integrin regulation and signaling. *Annu Rev Immunol* 2007;**25**:619–647.
3. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature* 2000;**407**:242–248.
4. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling—in control of vascular function. *Nat Rev Mol Cell Biol* 2006; **7**:359–371.
5. Bussolino F, Valdembri D, Caccavari F, Serini G. Semaphoring vascular morphogenesis. *Endothelium* 2006;**13**:81–91.
6. Hynes RO. Cell-matrix adhesion in vascular development. *J Thromb Haemost* 2007;**5**(Suppl. 1):32–40.
7. Serini G, Valdembri D, Bussolino F. Integrins and angiogenesis: a sticky business. *Exp Cell Res* 2006;**312**:651–658.
8. Borges E, Jan Y, Ruoslahti E. PDGF-receptor- and VEGF-receptor-2 bind to the 3 integrin through its extracellular domain. *J Biol Chem* 2000; **275**:39867–39873.
9. Soldi R, Mitola S, Strasly S, Defilippi P, Tarone G, Bussolino F. Role of $\alpha v\beta 3$ integrin in the activation of vascular endothelial growth factor receptor-2. *EMBO J* 1999;**18**:734–740.
10. Mahabeleshwar GH, Feng W, Phillips DR, Byzova TV. Integrin signaling is critical for pathological angiogenesis. *J Exp Med* 2006;**203**:2495–2507.
11. Mahabeleshwar GH, Feng W, Reddy K, Plow EF, Byzova TV. Mechanisms of integrin-vascular endothelial growth factor receptor cross-activation in angiogenesis. *Circ Res* 2007;**101**:570–580.
12. Masson-Gadais B, Houle F, Laferriere J, Huot J. Integrin avb3 requirement for VEGFR2- mediated activation of SAPK2/p38 and for Hsp90-dependent phosphorylation of focal adhesion kinase in endothelial cells activated by VEGF. *Cell Stress Chaperones* 2003;**8**:37–52.
13. Tian F, Zhu CH, Zhang XW, Xie X, Xin XL, Yi YH *et al*. Philinopside E, a new sulfated saponin from sea cucumber, blocks the interaction between kinase insert domain-containing receptor (KDR) and alphavbeta3 integrin via binding to the extracellular domain of KDR. *Mol Pharmacol* 2007;**72**:545–552.
14. 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. *Exp Cell Res* 2006;**312**:2973–2982.
15. Weis S, Lindquist JN, Barnes LA, Lutu-Fuga KM, Cui J, Wood MR *et al*. Cooperation between VEGF and $\beta 3$ integrin during cardiac vascular development. *Blood* 2007;**109**:1962–1970.
16. Silvestre JS, Thery C, Hamard G, Boddaert J, Aguilar B, Delcayre A *et al*. Lactadherin promotes VEGF-dependent neovascularization. *Nat Med* 2005;**11**:499–506.
17. Wijelath ES, Murray J, Rahman S, Patel Y, Ishida A, Strand K *et al*. Novel vascular endothelial growth factor binding domains of fibronectin enhance vascular endothelial growth factor biological activity. *Circ Res* 2002;**91**:25–31.
18. Wijelath ES, Rahman S, Namekata M, Murray J, Nishimura T, Mostafavi-Pour Z *et al*. Heparin-II domain of fibronectin is a vascular endothelial growth factor-binding domain: enhancement of VEGF biological activity by a singular growth factor/matrix protein synergism. *Circ Res* 2006;**99**:853–860.
19. Mitola S, Brenchio B, Piccinini M, Tertoolen L, Zammataro L, Breier G *et al*. Type I collagen limits VEGFR-2 signaling by a SHP2 protein-tyrosine phosphatase-dependent mechanism 1. *Circ Res* 2006;**98**: 45–54.
20. Jekely G, Sung HH, Luque CM, Rorth P. Regulators of endocytosis maintain localized receptor tyrosine kinase signaling in guided migration. *Dev Cell* 2005;**9**:197–207.
21. Seo DW, Li H, Guedez L, Wingfield PT, Diaz T, Salloum R *et al*. TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. *Cell* 2003;**114**:171–180.
22. Oh J, Seo DW, Diaz T, Wei B, Ward Y, Ray JM *et al*. Tissue inhibitors of metalloproteinase 2 inhibits endothelial cell migration through increased expression of RECK. *Cancer Res* 2004;**64**:9062–9069.
23. Seo DW, Li H, Qu CK, Oh J, Kim YS, Diaz T *et al*. Shp-1 mediates the antiproliferative activity of tissue inhibitor of metalloproteinase-2 in human microvascular endothelial cells. *J Biol Chem* 2006;**281**: 3711–3721.
24. Mattila E, Pellinen T, Nevo J, Vuoriluoto K, Arjonen A, Ivaska J. Negative regulation of EGFR signalling through integrin-alfa1beta1-mediated activation of protein tyrosine phosphatase TCPTP. *Nat Cell Biol* 2005; **7**:78–85.
25. Chen X, Abair TD, Ibanez MR, Su Y, Frey MR, Dise RS *et al*. Integrin alpha1beta1 controls reactive oxygen species synthesis by negatively regulating epidermal growth factor receptor-mediated Rac activation. *Mol Cell Biol* 2007;**27**:3313–3326.
26. Ling Y, Maile LA, Clemmons DR. Tyrosine phosphorylation of the beta3-subunit of the alphaVbeta3 integrin is required for membrane association of the tyrosine phosphatase SHP-2 and its further recruitment to the insulin-like growth factor I receptor. *Mol Endocrinol* 2003; **17**:1824–1833.
27. Zhang X, Groopman JE, Wang JF. Extracellular matrix regulates endothelial functions through interaction of VEGFR-3 and integrin $\alpha 5\beta 1$. *J Cell Physiol* 2005;**202**:205–214.
28. Wang JF, Zhang XF, Groopman JE. Stimulation of beta 1 integrin induces tyrosine phosphorylation of vascular endothelial growth factor receptor-3 and modulates cell migration. *J Biol Chem* 2001;**276**: 41950–41957.
29. Cascone I, Napione L, Maniero F, Serini G, Bussolino F. Stable interaction between alpha5beta1 integrin and Tie2 tyrosine kinase receptor regulates endothelial cell response to Ang-1. *J Cell Biol* 2005;**170**:993–1004.
30. Mitra SK, Schlaepfer DD. Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr Opin Cell Biol* 2006;**18**:516–523.
31. Bussolino F, Di Renzo MF, Ziche M, Bocchietto E, Olivero M, Naldini L *et al*. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. *J Cell Biol* 1992; **119**:629–641.
32. Trusolino L, Serini G, Cecchini G, Besati C, Ambesi-Impiombato FS, Marchisio PC *et al*. Growth factor-dependent activation of alphavbeta3 integrin in normal epithelial cells: implications for tumor invasion. *J Cell Biol* 1998;**142**:1145–1156.
33. Rahman S, Patel Y, Murray J, Patel KV, Sumathipala R, Sobel M *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 Biol* 2005;**6**:8.
34. Chung J, Yoon SO, Lipscomb EA, Mercurio AM. The Met receptor and alpha 6 beta 4 integrin can function independently to promote carcinoma invasion. *J Biol Chem* 2004;**279**:32287–32293.
35. Trusolino L, Bertotti A, Comoglio PM. A signaling adapter function for alpha6beta4 integrin in the control of HGF-dependent invasive growth. *Cell* 2001;**107**:643–654.
36. Nikolopoulos SN, Blaikie P, Yoshioka T, Guo W, Giancotti FG. Integrin beta4 signaling promotes tumor angiogenesis. *Cancer Cell* 2004;**6**: 471–483.
37. Brooks PC, Montgomery AM, Rosenfeld M, Reisfeld RA, Hu T, Klier G *et al*. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 1994;**79**:1157–1164.
38. Eliceiri BP, Klemke R, Stromblad S, Cheresh DA. Integrin alphavbeta3 requirement for sustained mitogen-activated protein kinase activity during angiogenesis. *J Cell Biol* 1998;**140**:1255–1263.

39. Hood JD, Frausto R, Kiosses WB, Schwartz MA, Cheresh DA. Differential alphav integrin-mediated Ras-ERK signaling during two pathways of angiogenesis. *J Cell Biol* 2003;**162**:933–943.
40. Sahni A, Francis CW. Stimulation of endothelial cell proliferation by FGF-2 in the presence of fibrinogen requires alphavbeta3. *Blood* 2004; **104**:3635–3641.
41. Toledo MS, Suzuki E, Handa K, Hakomori S. Effect of ganglioside and tetraspanins in microdomains on interaction of integrins with fibroblast growth factor receptor. *J Biol Chem* 2005;**280**:16227–16234.
42. Vlahakis NE, Young BA, Atakilit A, Hawkridge AE, Issaka RB, Boudreau N *et al*. Integrin alpha9beta1 directly binds to vascular endothelial growth factor (VEGF)-A and contributes to VEGF-A-induced angiogenesis. *J Biol Chem* 2007;**282**:15187–15196.
43. Vlahakis N, Young BA, Atakilit A, Sheppard D. The lymphangiogenic vascular enothelial growth factors VEGF-C and -D are ligand for the integrin alpha9beta1. *J Biol Chem* 2005;**280**:4544–4554.
44. Kajia K, Hirakawa S, Ma B, Drinnenberg I, Detmar M. Hepatocyte growth factor promotes lymphatic vessel formation and function. *EMBO J* 2005; **24**:2885–2895.
45. Hutchings H, Ortega N, Plouët J. Extracellular matrix-bound vascular endothelial growth factor promotes endothelial cell adhesion, migration, and survival through integrin ligation. *FASEB J* 2003;**17**: 1520–1522.
46. Ruhrberg C, Gerhardt H, Golding M, Watson R, Ioannidou S, Fujisawa H *et al*. Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. *Genes Dev* 2002;**16**:2684–2698.
47. Stalmans I, Ng YS, Rohan R, Fruttiger M, Bouche A, Yuce A *et al*. Arteriolar and venular patterning in retinas of mice selectively expressing VEGF isoforms. *J Clin Invest* 2002;**109**:327–336.
48. Dallabrida SM, Ismail N, Oberle JR, Himes BE, Rupnick MA. Angiopoietin-1 promotes cardiac and skeletal myocyte survival through integrins. *Circ Res* 2005;**96**:e8–e24.
49. Hu B, Jarzynka MJ, Guo P, Imanishi Y, Schlaepfer DD, Cheng SY. Angiopoietin 2 induces glioma cell invasion by stimulating matrix metalloprotease 2 expression through the alphavbeta1 integrin and focal adhesion kinase signaling pathway. *Cancer Res* 2006;**66**:775–783.
50. Carlson T, Feng Y, Maisonpierre P, Mrksich M, Morla A. Direct cell adhesion to the angiopoietins mediated by integrins. *J Biol Chem* 2001;**278**:26516–26525.
51. Imanishi Y, Hu B, Jarzynka MJ, Guo P, Elishaev E, Bar-Joseph I *et al*. Angiopoietin-2 stimulates breast cancer metastasis through the alpha(5)beta(1) integrin-mediated pathway. *Cancer Res* 2007;**67**: 4254–4263.
52. Kalluri R. Basement membranes: structures, assembly and role in tumor angiogenesis. *Nature Rev Cancer* 2003;**3**:422–433.
53. Wickstrom SA, Alitalo K, Keski-Oja J. An endostatin-derived peptide interacts with integrins and regulates actin cytoskeleton and migration of endothelial cells. *J Biol Chem* 2004;**279**:20178–20185.
54. Rehn M, Veikkola T, Kukk-Valdre E, Nakamura H, Ilmonen M, Lombardo C *et al*. Interaction of endostatin with integrins implicated in angiogenesis. *Proc Natl Acad Sci USA* 2001;**98**:1024–1029.
55. Sudhakar A, Sugimoto H, Yang C, Lively J, Zeisberg M, Kalluri R. Human tumstatin and human endostatin exhibit distinct antiangiogenic activities mediated by alpha v beta 3 and alpha 5 beta 1 integrins. *Proc Natl Acad Sci USA* 2003;**100**:4766–4771.
56. Wickstrom SA, Alitalo K, Keski-Oja J. Endostatin associates with integrin alpha5beta1 and caveolin-1, and activates Src via a tyrosyl phosphatase-dependent pathway in human endothelial cells. *Cancer Res* 2002;**62**: 5580–5589.
57. Maeshima Y, Sudhakar A, Lively JC, Ueki K, Kharbanda S, Kahn CR *et al*. Tumstatin, an endothelial cell-specific inhibitor of protein synthesis. *Science* 2002;**295**:140–143.
58. Sudhakar A, Nyberg P, Keshamouni VG, Mannam AP, Li J, Sugimoto H *et al*. Human alpha1 type IV collagen NC1 domain exhibits distinct antiangiogenic activity mediated by alpha1beta1 integrin. *J Clin Invest* 2005;**115**:2801–2810.
59. Magnon C, Galaup A, Mullan B, Rouffiac V, Bouquet C, Bidart JM *et al*. Canstatin acts on endothelial and tumor cells via mitochondrial damage initiated through interaction with alphavbeta3 and alphavbeta5 integrins. *Cancer Res* 2005;**65**:4353–4361.
60. Tarui T, Miles LA, Takada Y. Specific interaction of angiostatin with integrin alpha(v)beta(3) in endothelial cells. *J Biol Chem* 2001;**276**: 39562–39568.
61. Kruger RP, Aurandt J, Guan KL. Semaphorins command cells to move. *Nat Rev Mol Cell Biol* 2005;**6**:789–800.
62. Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. *Cell* 1998;**92**:735–745.
63. Kawasaki T, Kitsukawa T, Bekku Y, Matsuda Y, Sanbo M, Yagi T *et al*. A requirement for neuropilin-1 in embryonic vessel formation. *Development* 1999;**126**:4895–4902.
64. Shoji W, Isogai S, Sato-Maeda M, Obinata M, Kuwada JY. Semaphorin3a1 regulates angioblast migration and vascular development in zebrafish embryos. *Development* 2003;**130**:3227–3236.
65. Torres-Vazquez J, Gitler AD, Fraser SD, Berk JD, Van NP, Fishman MC *et al*. Semaphorin-plexin signaling guides patterning of the developing vasculature. *Dev Cell* 2004;**7**:117–123.
66. Serini G, Valdembri D, Zanivan S, Morterra G, Burkhardt C, Caccavari F *et al*. Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. *Nature* 2003;**424**:391–397.
67. Bates D, Taylor GI, Minichiello J, Farlie P, Cichowitz A, Watson N *et al*. Neurovascular congruence results from a shared patterning mechanism that utilizes Semaphorin3A and Neuropilin-1. *Dev Biol* 2003;**255**:77–98.
68. Vieira JM, Schwarz Q, Ruhrberg C. Selective requirements for NRP1 ligands during neurovascular patterning. *Development* 2007;**134**: 1833–1843.
69. Chia R, Achilli F, Festing MF, Fisher EM. The origins and uses of mouse outbred stocks. *Nat Genet* 2005;**37**:1181–1186.
70. Ito T, Kagoshima M, Sasaki Y, Li C, Udaka N, Kitsukawa T *et al*. Repulsive axon guidance molecule Sema3A inhibits branching morphogenesis of fetal mouse lung. *Mech Dev* 2000;**97**:35–45.
71. Damon DH. Vascular endothelial-derived semaphorin 3 inhibits sympathetic axon growth. *Am J Physiol Heart Circ Physiol* 2006;**290**: H1220–H1225.
72. Serini G, Ambrosi D, Giraudo E, Gamba A, Preziosi L, Bussolino F. Modeling the early stages of vascular network assembly. *EMBO J* 2003; **22**:1771–1779.
73. Vacca A, Scavelli C, Serini G, Di Pietro G, Cirulli T, Merchionne F *et al*. Loss of inhibitory semaphorin 3A (SEMA3A) autocrine loops in bone marrow endothelial cells of patients with multiple myeloma. *Blood* 2006;**108**:1661–1667.
74. Yonekura H, Sakurai S, Liu X, Migita H, Wang H, Yamagishi S *et al*. Placenta growth factor and vascular endothelial growth factor B and C expression in microvascular endothelial cells and pericytes. Implication in autocrine and paracrine regulation of angiogenesis. *J Biol Chem* 1999; **274**:35172–35178.
75. Virgintino D, Errede M, Robertson D, Girolamo F, Masciandaro A, Bertossi M. VEGF expression is developmentally regulated during human brain angiogenesis. *Histochem Cell Biol* 2003;**119**:227–232.
76. Tang N, Wang L, Esko J, Giordano FJ, Huang Y, Gerber HP *et al*. Loss of HIF-1alpha in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. *Cancer Cell* 2004;**6**:485–495.
77. Lee S, Chen TT, Barber CL, Jordan MC, Murdock J, Desai S *et al*. Autocrine VEGF signaling is required for vascular homeostasis. *Cell* 2007;**130**:691–703.
78. Gitler AD, Lu MM, Epstein JA. PlexinD1 and semaphorin signaling are required in endothelial cells for cardiovascular development. *Dev Cell* 2004;**7**:107–116.
79. Herzog Y, Guttmann-Raviv N, Neufeld G. Segregation of arterial and venous markers in subpopulations of blood islands before vessel formation. *Dev Dyn* 2005;**232**:1047–1055.
80. Kusy S, Funkelstein L, Bourgais D, Drabkin H, Rougon G, Roche J *et al*. Redundant functions but temporal and regional regulation of two alternatively spliced isoforms of semaphorin 3F in the nervous system. *Mol Cell Neurosci* 2003;**24**:409–418.
81. Narazaki M, Tosato G. Ligand-induced internalization selects use of common receptor neuropilin-1 by VEGF165 and semaphorin3A. *Blood* 2006;**107**:3892–3901.
82. Guttmann-Raviv N, Shraga-Heled N, Varshavsky A, Guimaraes-Sternberg C, Kessler O, Neufeld G. Semaphorin-3A and semaphorin-3F work together to repel endothelial cells and to inhibit their survival by induction of apoptosis. *J Biol Chem* 2007;**282**:26294–26305.
83. Gu C, Yoshida Y, Livet J, Reimert DV, Mann F, Merte J *et al*. Semaphorin 3E and plexin-D1 control vascular pattern independently of neuropilins. *Science* 2005;**307**:265–268.
84. Chauvet S, Cohen S, Yoshida Y, Fekrane L, Livet J, Gayet O *et al*. Gating of Sema3E/PlexinD1 signaling by neuropilin-1 switches axonal repulsion to attraction during brain development. *Neuron* 2007;**56**: 807–822.
85. Christensen C, Ambartsumian N, Gilestro G, Thomsen B, Comoglio P, Tamagnone L *et al*. Proteolytic processing converts the repelling signal

Sema3E into an inducer of invasive growth and lung metastasis. *Cancer Res* 2005;**65**:6167–6177.
86. Roodink I, Raats J, van der Zwaag B, Verrijp K, Kusters B, van Bokhoven H *et al*. Plexin D1 expression is induced on tumor vasculature and tumor cells: a novel target for diagnosis and therapy? *Cancer Res* 2005;**65**:8317–8323.
87. Toyofuku T, Yabuki M, Kamei J, Kamei M, Makino N, Kumanogoh A *et al*. Semaphorin-4A, an activator for T-cell-mediated immunity, suppresses angiogenesis via Plexin-D1. *EMBO J* 2007;**26**:1373–1384.
88. Acevedo LM, Barillas S, Weis SM, Gothert JR, Cheresh DA. Semaphorin 3A suppresses VEGF-mediated angiogenesis yet acts as a vascular permeability factor. *Blood* 2008;**111**:2674–2680.
89. Kessler O, Shraga-Heled N, Lange T, Gutmann-Raviv N, Sabo E, Baruch L *et al*. Semaphorin-3F is an inhibitor of tumor angiogenesis. *Cancer Res* 2004;**64**:1008–1015.
90. Potiron VA, Sharma G, Nasarre P, Clarhaut JA, Augustin HG, Gemmill RM *et al*. Semaphorin SEMA3F affects multiple signaling pathways in lung cancer cells. *Cancer Res* 2007;**67**:8708–8715.
91. Bielenberg DR, Hida Y, Shimizu A, Kaipainen A, Kreuter M, Kim CC *et al*. Semaphorin 3F, a chemorepulsant for endothelial cells, induces a poorly vascularized, encapsulated, nonmetastatic tumor phenotype. *J Clin Invest* 2004;**114**:1260–1271.
92. Kashiwagi H, Shiraga M, Kato H, Kamae T, Yamamoto N, Tadokoro S *et al*. Negative regulation of platelet function by a secreted cell repulsive protein, semaphorin 3A. *Blood* 2005;**106**:913–921.
93. Lepelletier Y, Smaniotto S, Hadj-Slimane R, Villa-Verde DM, Nogueira AC, Dardenne M *et al*. Control of human thymocyte migration by Neuropilin-1/Semaphorin-3A-mediated interactions. *Proc Natl Acad Sci USA* 2007;**104**:5545–5550.
94. Toyofuku T, Yoshida J, Sugimoto T, Zhang H, Kumanogoh A, Hori M *et al*. FARP2 triggers signals for Sema3A-mediated axonal repulsion. *Nat Neurosci* 2005;**8**:1712–1719.
95. Banu N, Teichman J, Dunlap-Brown M, Villegas G, Tufro A. Semaphorin 3C regulates endothelial cell function by increasing integrin activity. *FASEB J* 2006;**20**:2150–2152.
96. Campbell ID, Ginsberg MH. The talin–tail interaction places integrin activation on FERM ground. *Trends Biochem Sci* 2004;**29**:429–435.
97. Luo BH, Springer TA. Integrin structures and conformational signaling. *Curr Opin Cell Biol* 2006;**18**:579–586.
98. Kinbara K, Goldfinger LE, Hansen M, Chou FL, Ginsberg MH. Ras GTPases: integrins' friends or foes? *Nat Rev Mol Cell Biol* 2003;**4**:767–776.
99. Furuhjelm J, Peranen J. The C-terminal end of R-Ras contains a focal adhesion targeting signal. *J Cell Sci* 2003;**116**:3729–3738.
100. Self AJ, Caron E, Paterson HF, Hall A. Analysis of R-Ras signalling pathways. *J Cell Sci* 2001;**114**:1357–1366.
101. Goldfinger LE, Ptak C, Jeffery ED, Shabanowitz J, Hunt DF, Ginsberg MH. RLIP76 (RalBP1) is an R-Ras effector that mediates adhesion-dependent Rac activation and cell migration. *J Cell Biol* 2006;**174**:877–888.
102. Oinuma I, Ishikawa Y, Katoh H, Negishi M. The Semaphorin 4D receptor Plexin-B1 is a GTPase activating protein for R-Ras. *Science* 2004;**305**:862–865.
103. Oinuma I, Katoh H, Negishi M. Semaphorin 4D/Plexin-B1-mediated R-Ras GAP activity inhibits cell migration by regulating beta(1) integrin activity. *J Cell Biol* 2006;**173**:601–613.
104. Basile JR, Barac A, Zhu T, Guan KL, Gutkind JS. Class IV semaphorins promote angiogenesis by stimulating Rho-initiated pathways through plexin-B. *Cancer Res* 2004;**64**:5212–5224.
105. Conrotto P, Valdembri D, Corso S, Serini G, Tamagnone L, Comoglio PM *et al*. Sema4D induces angiogenesis through Met recruitment by Plexin B1. *Blood* 2005;**105**:4321–4329.
106. Basile JR, Gavard J, Gutkind JS. Plexin-B1 utilizes RhoA and Rho kinase to promote the integrin-dependent activation of Akt and ERK and endothelial cell motility. *J Biol Chem* 2007;**282**:34888–34895.
107. Swiercz JM, Worzfeld T, Offermanns S. ERBB-2 and met reciprocally regulate cellular signaling via plexin-B1. *J Biol Chem* 2008;**283**:1893–1901.
108. Zamir E, Geiger B. Molecular complexity and dynamics of cell-matrix adhesions. *J Cell Sci* 2001;**114**:3583–3590.
109. Avraham HK, Lee TH, Koh Y, Kim TA, Jiang S, Sussman M *et al*. Vascular endothelial growth factor regulates focal adhesion assembly in human brain microvascular endothelial cells through activation of the focal adhesion kinase and related adhesion focal tyrosine kinase. *J Biol Chem* 2003;**278**:36661–36668.
110. Le Boeuf F, Houle F, Sussman M, Huot J. Phosphorylation of focal adhesion kinase (FAK) on Ser732 is induced by rho-dependent kinase and is essential for proline-rich tyrosine kinase-2-mediated phosphorylation of FAK on Tyr407 in response to vascular endothelial growth factor. *Mol Biol Cell* 2006;**17**:3508–3520.
111. Eliceiri BP, Puente XS, Hood JD, Stupack DG, Schlaepfer DD, Huang XZ *et al*. Src-mediated coupling of focal adhesion kinase to integrin alpha(v)beta5 in vascular endothelial growth factor signaling. *J Cell Biol* 2002;**157**:149–160.
112. Julien F, Bechara A, Fiore R, Nawabi H, Zhou H, Hoyo-Becerra C *et al*. Dual functional activity of semaphorin 3B is required for positioning the anterior commissure. *Neuron* 2005;**48**:63–75.
113. Ikeda S, Ushio-Fukai M, Zuo L, Tojo T, Dikalov S, Patrushev NA *et al*. Novel role of ARF6 in vascular endothelial growth factor-induced signaling and angiogenesis. *Circ Res* 2005;**96**:467–475.
114. Arias-Salgado AR, Haj F, Dubois C, Moran B, Kasirer-Friede A, Furie BC *et al*. PTB-1B is essential positive regulator of platelet integrin signals. *J Cell Biol* 2005;**170**:837–845.