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Review

Vascular integrins: pleiotropic adhesion and signaling molecules in vascular homeostasis and angiogenesis

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Abstract. New blood vessel formation, a process referred to as angiogenesis, is essential for embryonic development and for many physiological and pathological processes during postnatal life, including cancer progression. Endothelial cell adhesion molecules of the integrin family have emerged as critical mediators and regulators of angiogenesis and vascular homeostasis. Integrins provide the physical interaction with the extracellular matrix necessary for cell adhesion, migration and positioning, and induction of signaling events essential for cell survival, proliferation and differentiation. Antagonists of integrin $\alpha V\beta 3$ suppress angiogenesis in many experimental models and are currently tested in clinical trials for their therapeutic efficacy against angiogenesis-dependent diseases, including cancer. Furthermore, interfering with signaling pathways downstream of integrins results in suppression of angiogenesis and may have relevant therapeutic implications. In this article we review the role of integrins in endothelial cell function and angiogenesis. In the light of recent advances in the field, we will discuss their relevance as a therapeutic target to suppress tumor angiogenesis.

Key words. Angiogenesis; apoptosis; cancer; therapy; endothelial cell; signaling; integrin; cell adhesion.

Introduction

Research in vascular biology, angiogenesis and lymphangiogenesis has experienced an enormous development over the past 2 decades. Thanks to contributions from physiology, developmental biology, mouse genetics, cell and molecular biology, experimental pathology and drug development, we have gained a detailed insight into many of the cellular and molecular events that mediate and regulate blood vessel formation. A main reason for the enormous interest in vascular biology and angiogenesis is due to its potential for therapeutic interventions in pathological conditions such as cancer, chronic inflammation and cardiovascular diseases [1, 2]. Integrin adhesion receptors, together with vascular growth factors and their receptors, are well-studied mediators of angiogenesis. A significant amount of experimental data indicates that vascular integrins may represent suitable targets for therapeutic inhibition of angiogenesis. Preclinical studies have demonstrated that integrin antagonists (i.e. low molecular weight inhibitors and function-blocking antibodies) can efficiently suppress angiogenesis and inhibit progression of diseases that depend on angiogenesis, including cancer. Antagonists of integrin $\alpha V\beta 3$ are currently being tested in clinical trials for their therapeutic safety and effects as anticancer agents.

Recent developments have questioned the role of $\alpha V\beta 3$ integrin as a positive mediator of angiogenesis, and the mechanism by which $\alpha V\beta 3$-antagonists suppress angiogenesis. A reevaluation of the role of integrins, in particular $\alpha V\beta 3$, as regulators of angiogenesis and the strate-

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gies aimed at their therapeutic modulation is highly necessary. In this article we will review experimental evidence for the role of integrins in regulating endothelial cell function and signaling events and will provide critical considerations for the development of new antiangiogenic therapeutic strategies.

Vasculogenesis, angiogenesis and lymphangiogenesis

During embryonic development, blood vessels form through two distinct mechanisms: vasculogenesis and angiogenesis. During vasculogenesis, a mesoderm-derived hematopoietic and vascular stem cell, the hemangioblast, gives rise to a committed endothelial cell progenitor, the angioblast. In turn, the angioblast generates differentiated endothelial cells to form a primitive vascular plexus [3–5], which is then remodeled through sprouting, trimming, intussusception and differentiation into a mature vascular system with hierarchically defined functional compartments (e.g. large vs. small vessels, arteries vs. veins vs. capillaries) in a process globally referred to as angiogenesis [6]. An increasing number of molecules are found to contribute to the development of the mature vascular system. Most of these molecules can be classified in six main classes: (i) growth factors and their receptors [e.g. vascular endothelial growth factor (VEGF) A, B, C and D and VEGF-R 1, 2 and 3; fibroblast growth factors (FGFs) and FGF-Rs] [7, 8]; (ii) remodeling and morphogenic factors (e.g. angiopoietins/TIEs and Eph/ephrins) [9, 10]; (iii) adhesion receptors and matrix proteins (e.g. integrins, cadherins, fibronectin) [11–13]; (iv) matrix-degrading enzymes (e.g. matrix metalloproteinases (MMPs)) [14]; (v) signaling molecules (e.g. Raf and mitogen-activated protein kinase (MAPK), protein kinase A (PKA), Rho-family GTPases, protein kinase B (PKB/Akt)) [15–18]; (vi) transcription factors and regulators [e.g. hypoxia-inducible factor (HIF) -1α, Id1/3, nuclear factor (NF)-κB] [19–21] and homeobox gene products (e.g. Hox D3 and B3) [22, 23]. At the cellular level, these molecules work in concert to promote and regulate the main endothelial functions and events required for angiogenesis: cell proliferation, survival, migration, matrix remodeling, tube formation and differentiation [24]. This vascular maturation process also involves the recruitment of perivascular cells, the pericytes [25], which confer mechanical stability and contractility to the vessel wall, and provide essential factors for endothelial cell survival, such as VEGF and angiopoietin-1 [26, 27]. During postnatal angiogenesis quiescent endothelial cells need to 'escape' from the stabilizing effects of the vessel wall in order to respond to angiogenic factors and sprout into the surrounding tissue. The exact mechanism by which this escape is achieved remains to be elucidated. The observation that angiopoietin-2 is expressed very early at sites of angiogenesis, and that it can act as a natural antagonist of angiopoietin-1 on endothelial cells, suggests the possibility that expression of angiopoietin-2 may loosen the interactions between endothelial cells and the vessel wall, and renders endothelial cells responsive to VEGF [28, 29].

In contrast to physiological angiogenesis (as it occurs, for example, during tissue repair or ovarian follicle maturation), tumor vessels do not undergo full maturation. They frequently display incomplete endothelial cell lining of the vessels with many tumor cells in direct contact with the circulation [30], they show limited and disorganized recruitment of pericytes [31], remain highly permeable and unstable and fail to generate a hierarchically branched structure [32]. Postnatal blood vessel formation was long considered to be entirely mediated by endothelial cell sprouting from preexisting vessels. This paradigm was recently challenged by the observation that bone-marrow-derived endothelial cell progenitors circulate in the blood of healthy individuals and animals in response to tissue ischemia [33–35]. Circulating endothelial cell progenitors can incorporate into sites of angiogenesis, including the tumor vasculature, and contribute to augment neovascularization possibly by in situ differentiation into mature endothelial cells [36, 37].

In contrast to the extensive knowledge on blood vessel formation, much less is known about lymphangiogenesis, the process that leads to lymphatic vessel formation [38]. The key function of lymphatic vessels is to drain extracellular fluid, pathogens and cells from the interstitial space back to lymph nodes ('immunological surveillance') and from there into the venous circulation. Insufficient lymphatic flow causes lymphedema, while excessive lymphatic vessels around tumors facilitate tumor cell spreading and metastasis formation [39]. The recent discovery that VEGF-C and VEGF-D and their receptor VEGFR-3 promote lymphangiogenesis has greatly boosted research on lymphangiogenesis and has opened new therapeutic perspectives for the treatment of lymphedema and the inhibition of tumor cell spreading [40, 41].

Integrin adhesion molecules

Integrins are heterodimeric cell surface molecules consisting of two noncovalently associated glycoproteins, α and β. There are to date 18 α and 8 β subunits which associate to form at least 24 different heterodimers (fig. 1) [42, 43]. α subunits consist of 1000–1200 amino acids and some contain a so-called inserted domain (I domain) near the amino terminal end. The β subunits are shorter (~700–800 amino acids). The cytoplasmic domains of both α and β subunits are short (30–40 and 40–50 residues, respectively), with the exception of the β4 subunit (over 1000 residues long). Integrins are the main receptors for extracellular matrix (ECM) proteins, such as

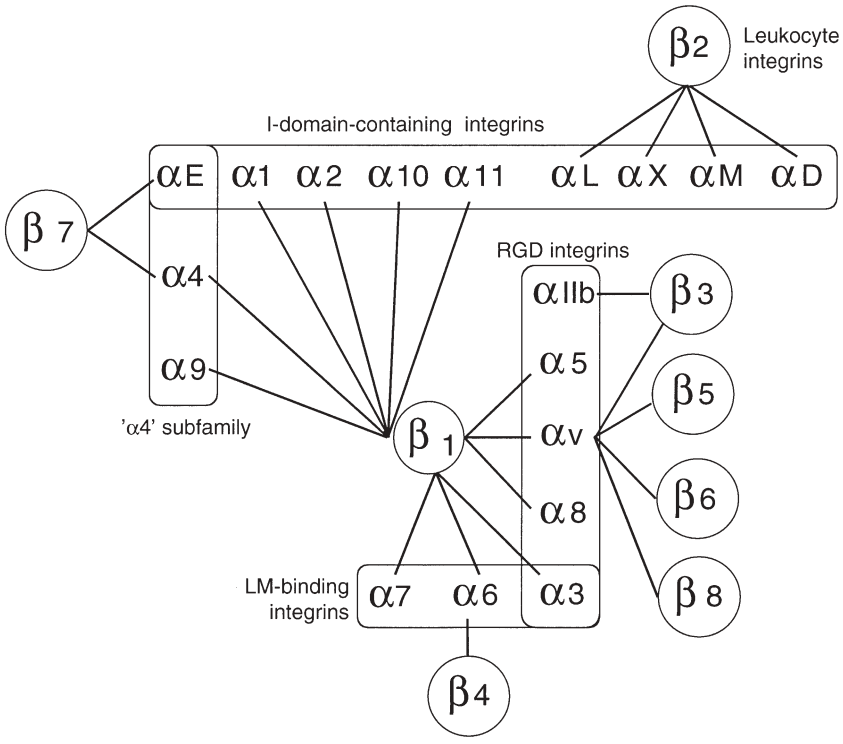

Figure 1. The integrin family of adhesion receptors. There are to date 18α and 8β known mammalian subunits which assemble to form 25 different heterodimers. Integrins are the main receptors for ECM proteins, but they can also bind cellular counterreceptors, soluble molecules and pathogens (e.g. viruses and bacteria). Major integrin subclasses (leukocyte integrins, I-domain-containing integrins, 'α4' subfamily, laminin-binding integrins and RGD integrins) are indicated.

fibronectin, laminin, collagens, vitronectin and thereby the main mediators of cell adhesion to the ECM. With a few exceptions, integrin-ECM interactions are highly promiscuous. First, different integrins can bind the same ligand. For example, integrins $\alpha3\beta1$, $\alpha4\beta1$, $\alpha5\beta1$, $\alpha8\beta1$, $\alpha V\beta1$, $\alpha V\beta3$, $\alpha V\beta5$, $\alpha V\beta6$, $\alpha4\beta7$, $\alpha V\beta8$ can all bind fibronectin. The exact reason for the promiscuous ligand-binding properties of most integrins is still not completely understood. There is evidence that upon binding to the same ECM protein (e.g. fibronectin) different integrins can mediate different signaling events and functions. For example, integrin $\alpha5\beta1$ binding to fibronectin suppresses cell migration [44], while $\alpha V\beta6$ binding to fibronectin promotes it [45]. Furthermore, integrins with overlapping ligand-binding function may have different spatiotemporal patterns of expression, thus reducing redundancy. On the other side, one specific integrin can bind multiple ligands. For example, integrin $\alpha V\beta3$ can bind fibronectin, vitronectin, thrombospondin, tenascin-C, fibrin, von Willenbrand factor, osteopontin and denatured collagen I [42]. This may be advantageous when the cellular function mediated by a particular integrin (e.g. migration) is more important than the nature of the extracellular ligand promoting it. Besides acting as ECM receptors, some integrins (in particular $\beta2$ integrins) can bind to cell surface counterreceptors, such as the intercellular adhesion molecules (ICAMs), or soluble plasma molecules (e.g. fibrinogen). Integrin-dependent cell-cell adhesion events are particularly important for leukocyte adhesion to the endothelium, transendothelial migration at sites of inflammation, and platelet aggregation and thrombus formation during hemostasis [46].

Structural basis of integrin activation and ligand binding

Integrins exist in at least two distinct functional states: an 'inactive' (i.e. non-adhesion-promoting) and an 'active' (adhesion-promoting) state. Integrin activation is very rapid (<1 s) and involves increased affinity ('affinity maturation'), clustering and association with the cytoskeleton ('avidity maturation') [47]. Since the identification of integrins in the mid-eighties, the elucidation of the molecular basis of their activation and ligand-binding function has been an area of intense research. Our current understanding of integrin activation and ligand-binding function stems from the functional mutational analysis of the extracellular α and β domains, the study of the role of the α and β cytoplasmic domains in modulating integrin ligand-binding activity, and the resolution of the three-dimensional structure of the I domain (of the αM, αL and

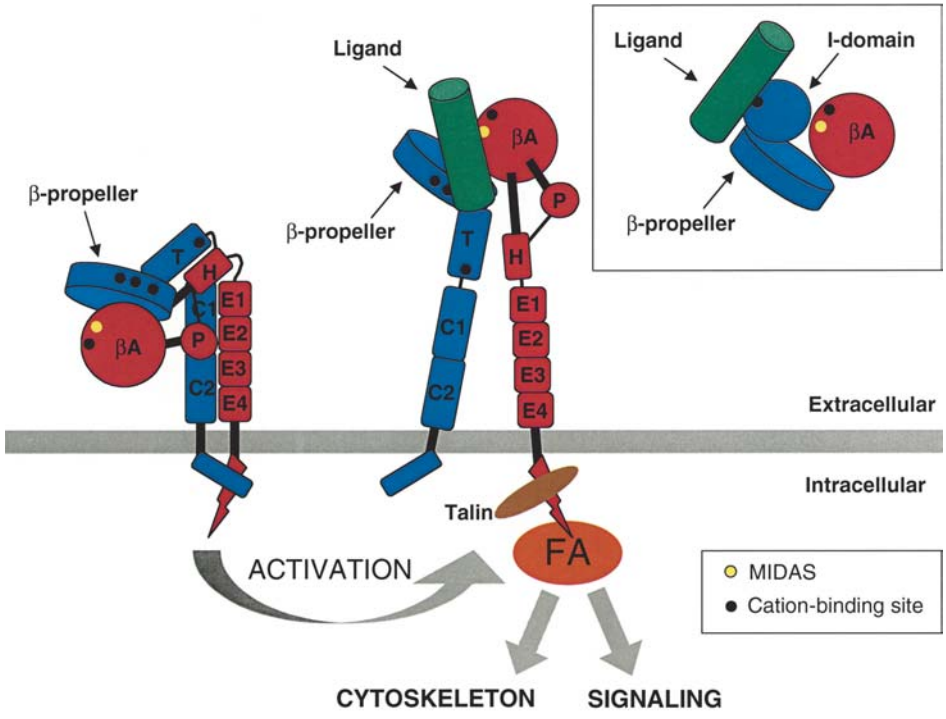

Figure 2. Proposed model of integrin structure and activation. Integrins consist of a 'head' region and two 'legs'. The heads contain a 'β-propeller' domain (α subunit) and a 'propeller-like' domain (β subunit). A large interface between these two domains allows for stable association of a heterodimer. In some α subunits, an I domain is inserted into the β-propeller domain and participates in promoting ligand binding (insert). The legs consist of tightly packed domains that form a rigid stalk with a flexible hinge region. Five cation-binding sites are located within the head, while a sixth one is present on the α subunit near the hinge region. The current model of integrin function proposes that the inactive, non-ligand-bound integrin is bent at the 'knee' region, with the ligand-binding pocket in a low-affinity conformation. The association of the α and β cytoplasmic domains retains the integrin in the resting state. Disruption of the interaction between the α and β cytoplasmic domains triggers an inside-out conformational change leading to receptor activation. The binding of talin to the β cytoplasmic domain and disruption of the interaction between the α and β cytoplasmic domains has been demonstrated in vitro. The active receptor is 'stretched', has the ligand-binding pocket in a high-affinity conformational state and altered coordination site in the MIDAS. Additional cytoplasmic proteins are recruited to form focal adhesions (FA). T, thigh domain; C1-2, Calf domain-1 and -2; βA, propeller-like domain; H, hybrid domain, P, plexin-semaphorin-integrin domain; E1-4, epidermal growth factor (EGF)-like repeats 1–4. The cation binding sites are shown as black dots, the MIDAS is shown as a yellow dot. Based on [53–55, 60–62]. The α subunit is shown in blue, the β subunit in red.

α2 subunits) [48–52], of the extracellular domain of αVβ3 [53, 54] and of the cytoplasmic tail of αIIbβ3 [55–58]. We are now beginning to appreciate the structural features of integrins and the conformational changes that regulate affinity and ligand binding [55, 59–63]. Here we will briefly summarize the current view of integrin structure and the putative model of integrin activation (see fig. 2).

The overall shape of the heterodimer is that of a large 'head' on two 'legs'. The head consists of a seven-blade 'β-propeller' domain on the α subunit, and a 'propeller-like' domain on the β subunit (the βA domain). A large interface between these two domains allows for stable association of the α and β subunits. In some α subunits, an additional propeller-like domain is inserted into the β-propeller domain between the second and third blade (αA or I domain). The I domain exists in a closed or open conformation. The latter promotes ligand binding for integrins that express the I domain. The 'legs' consist of tightly packed domains that form a rigid stalk with a flexible hinge region near the head. Five cation binding sites are located within the head, while a 6th one is present on the α subunit near the hinge region. One cation binding site on the top of the β propeller is known as the metal ion-dependent adhesion site (MIDAS) and participates in ligand binding. The ligand-binding pocket is located between the β-propeller domain of the α subunit and the βA domain of the β subunit. Structure-function analysis of recombinant soluble integrin αVβ3 revealed that the inactive, non-ligand-bound integrin, has a 'bent' conformation. Upon addition of manganese or addition of a high-affinity ligand, the recombinant soluble αVβ3 integrin assumes a 'stretched' conformation. At the cell surface an inactive integrin would have the ligand-binding pocket in its low-affinity conformation and its 'head' domain facing down toward the membrane, while an active integrin would 'stand up' with the ligand-binding pocket in a high-affinity conformation easily accessible to ligands.

Although there is indirect evidence that conformational movements may occur in cell surface integrins, definitive demonstration that the bent and stretched forms may exist at the cell surface, as well as their physiological relevance, remain to be demonstrated. Furthermore, it should be stressed here that although there is compelling evidence indicating that the bent and stretched integrins correspond to the inactive and active forms, additional, still undisclosed mechanisms may be at play in controlling integrin activation.

The association of the α and β cytoplasmic domains retain integrins in their resting state. Disruption of this interaction has emerged as a critical physiological event that triggers integrin activation. How this disruption, and hence integrin activation is initiated in a living cell remains unclear. Talin binding to the β cytoplasmic domain has been demonstrated in vitro and was associated with conformational changes in the $\alpha\beta$ cytoplasmic domains consistent with integrin activation. Thus, talin may be considered a likely candidate for physiological integrin activation in vivo. How disruption of the association of the $\alpha\beta$ cytoplasmic domains propagate to the extracellular domain of the receptors and induces integrin activation ('inside out' signaling) remains to be determined. A proposed model is that upon unclasping of the $\alpha\beta$ cytoplasmic domains, there is a slight separation of the $\alpha\beta$ transmembrane regions and extracellular stalks which causes stretching of the integrin and rearrangement of the 'head domains' into a high-affinity ligand-binding conformation. Conformational changes in the head domain and repositioning of one cation coordination site of the MIDAS from the β subunit toward the arginine-glycine-aspartic acid (RGD) site on the ligand have been demonstrated experimentally. Also, it is not clear whether and how activated integrins can be inactivated at the cell surface.

Once activated, integrins rapidly cluster and recruit many additional structural (e.g. vinculin, α-actinin), adaptor (e.g. Shc, Cas, Crk) and signaling [e.g. focal adhesion kinase (FAK), paxillin and c-src] proteins to form characteristic focal structures. Depending on their size and subcellular localization, these structures are named focal contacts, focal adhesions or fibrillar adhesion [64, 65]. Integrins and focal adhesions propagate tensional forces between the ECM and the cytoskeleton necessary to stabilize cell adhesion and initiate signaling cascades [66].

Integrin-mediated signaling

Integrin-mediated signaling events are essential for cell spreading, migration, survival, proliferation and differentiation [67, 68]. Integrins, however, have no intrinsic enzymatic activity. Signal transduction upon ligand binding depends on the recruitment of crucial signaling molecules, in particular, protein kinases (e.g. src family kinases, PKA or FAK), lipid kinases [i.e. phosphatidylinositol 3-kinase (PI3-K)], small GTPases (e.g. Rho and Ras families), and phosphatases (e.g. Shp-2) [66, 67]. Many signaling pathways activated by integrins are also activated by growth factor receptors. The cross-talk between integrins and growth factor receptors is believed to provide enhanced specificity and control over many cellular events, compared with signaling from a single receptor class. A number of excellent reviews on integrin signaling and growth factor receptor cross-talk have recently been published [68–72]. Here we will limit our review to the main signaling pathways downstream of integrins that are directly relevant to endothelial cells in angiogenesis (see fig. 3).

MAPK

Both growth factor receptors and integrins can activate the Ras-MAPK signaling pathway that culminates in MAPKs [e.g. extracellular regulated kinase (ERK), Jun N-terminal kinase (JNK)] translocation into the nucleus and regulation of gene expression and of several aspects of cell behavior, such as proliferation, survival and migration. In normal cells, efficient MAPK activation can be achieved only if both growth factor receptors and integrins contribute to the stimulation of this pathway. Aberrant activation of one type of these receptors, as seen for example in some cancers, can overcome the need for a combined input for MAPK activation, leading to their uncontrolled stimulation.

Stimulation of ERK by endothelial-cell integrins can be achieved through different mechanisms, requiring activation of either FAK, or the Src-family kinases Fyn and Yes. In one case, upon recruitment by integrins, FAK autophosphorylates at ^{397}Y and can thus associate with Src or Fyn, which further phosphorylates it at ^{925}Y, allowing binding of the complex Grb2-Sos and subsequent activation of the Ras cascade to ERK [73, 74]. The second pathway is stimulated by some β1 and αV integrins that activate Fyn or Yes through a yet undefined mechanism, promoting Shc binding to the Src-family kinase SH3 domain, its tyrosine phosphorylation and subsequent recruitment of the complex Grb2-Sos [75, 76]. Activated ERK enters the nucleus and phosphorylates the ternary complex factors, thus promoting transcription [77]. Probably both pathways contribute to ERK activation by integrins, but it is not yet clear how they are coordinated.

Angiogenesis in the chorio allantoid membrane (CAM) model requires two waves of ERK activation, the first one being dependent on FGF-2, and the second one on αVβ3 integrin ligation. It was shown that inhibition of the late, sustained phase of ERK activity by αVβ3 antagonists or by chemical inhibitors prevented new blood vessel formation without affecting preexisting vessels, thus indi-

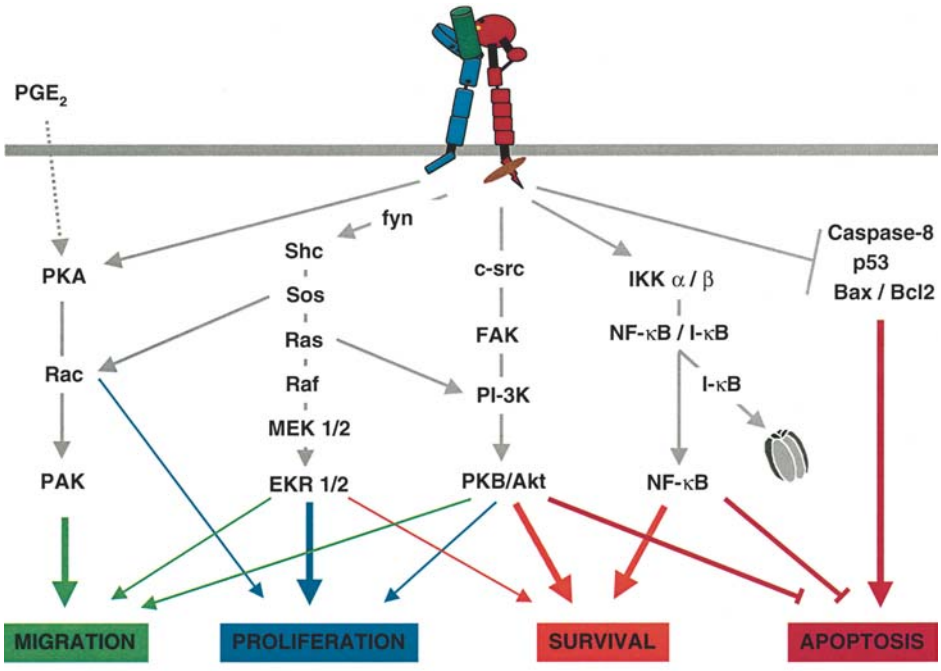

Figure 3. The major signaling pathways activated or modulated by integrins. Integrin ligation activates four major signaling pathways relevant to angiogenesis: the Rac GTPase, the Ras-Raf-MEK, PI3-K-PKB/Akt and NF-κB pathways. These pathways generate signals that are essential for endothelial cell migration, proliferation and survival.

cating the importance of integrin-dependent ERK activation for angiogenesis [78]. Whether the sustained activation of ERK in response to FGF-2 and $\alpha V\beta 3$ ligation also occurs during other situations of angiogenesis, or whether it is restricted to the CAM model remains to be addressed. JNK is an important modulator of endothelial cell function and has often been implicated in opposing cellular responses [79]. While there is considerable evidence that JNK activation promotes endothelial cell apoptosis, there are also reports demonstrating that under certain circumstances, JNK activation promotes cell survival and proliferation. For example, endothelial cell exposure to lipopolysaccharide [80], colchicin [81], vascular endothelial growth inhibitor [82] or homocysteine [83] results in JNK activation and JNK-dependent apoptosis. In contrast, integrin-mediated activation of JNK is rather associated with induction of cell proliferation and survival. In this respect, it was shown that in primary endothelial cells, integrin-mediated JNK activation promoted cell cycle progression through AP-1-dependent gene expression and required FAK association with Src and p130Cas, and recruitment of the adaptor protein Crk [84]. Consistent with these findings, fibronectin-dependent survival of fibroblasts and endothelial cells was shown to involve FAK-dependent activation of JNK [85]. Also, shear stress-mediated endothelial cell survival was shown to occur through multiple signaling pathways [86], including integrin-mediated and FAK-dependent activation of JNK and p38 [87]. The exact reasons why JNK activation by different stimuli can lead to divergent endothelial cell responses, and whether JNK activation by integrins may have a rather antiapototic effect, remain open questions.

NF-κB

Nuclear factor κB (NF-κB) is a ubiquitously expressed transcription factor that plays a central role in modulating the immune response and in promoting inflammation and cancer [88, 89]. NF-κB promotes cell survival in response to pro-apoptotic stimuli by inducing expression of anti-apoptotic molecules, such as the inhibitors of apoptosis (IAPs) [88]. NF-κB activity is also critical to the regulation of endothelial cell function in inflammation and angiogenesis [21]. Suppression of NF-κB expression in endothelial cells inhibits tubular morphogenesis in vitro [90], while pharmacological inhibition of NF-κB in vivo suppresses retinal neovascularization in a murine model of ischemic retinopathy [91]. While NF-κB activation was originally reported in response to inflammatory cytokines, such as TNF, or bacterial lipopolysaccharide, recent evidence indicates that integrins can also activate NF-κB. Ligation of endothelial cell integrin $\alpha V\beta 3$ by osteopontin was shown to protect endothelial cells against serum withdrawal-induced apoptosis through the activation of NF-κB [92]. NF-κB-dependent expression of osteoprotogerin was subsequently reported to mediate this NF-κB effect [93]. Using a genome-wide screening ap-

proach, it was recently reported that $\alpha5\beta1$-mediated adhesion to fibronectin, but not $\alpha2\beta1$-mediated adhesion to laminin, activates an NF-κB-dependent gene expression program important for angiogenesis and inflammation [94]. Inhibition of NF-κB activation in vivo by retroviral-mediated delivery of an inhibitor-κB (I-κB) superrepressor blocked FGF-2-induced angiogenesis in the Matrigel plug assay [94].

Rho-family GTPases

Rho, Rac and cdc42 are small GTPases that participate in the regulation of actin polymerization, leading to the formation of stress fibers, focal adhesions, lamellipodia and filopodia [95]. Activation of Rho, Rac and cdc42 requires GTP loading, which is tightly controlled by guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs) [96–97]. Rho protein activation also requires localization to the cell membrane, an event that depends on isoprenylation at the carboxyl-terminal end [98]. Activated Rho GTPases bind to effector proteins that regulate actin turnover and polymerization, as well as cell proliferation and gene expression [99, 100]. Integrin ligation activates Rho proteins by promoting GTP loading and inducing translocation from the cytosol to the membrane [97]. Rac activity was recently shown to be essential for endothelial cell migration and angiogenesis [101, 102]. The Rac effector protein p21 activated kinase 1 (PAK1), a serine/threonine kinase that modulates cell contraction and thereby cell migration [103], is a critical mediator of the angiogenic activity of Rac. Inhibition of Rac or PAK1 function by dominant-negative constructs in endothelial cells in vivo inhibited growth factor-induced angiogenesis [102, 103]. Strikingly, Rac activation by $\alpha V\beta3$ requires COX-2 activity and prostaglandin (PG) E_2 synthesis, while activation by $\alpha5\beta1$ does not [102, 104] (see below). Recently it was also shown that activated Rac promotes recruitment of high-affinity integrins $\alpha V\beta3$ to lamellipodia [105]. This integrin translocation to the lamellipodia promotes formation of new adhesions at the leading edge which are essential for directional cell migration. Furthermore, integrin-specific activation of Rac by $\alpha5\beta1$ is required for endothelial cell proliferation on fibronectin [106]. Active Rac promotes cyclin D1 synthesis through shc, FAK, SOS and phosphatidylinositol 3-kinase (PI-3K)-dependent signaling. Thus, integrin-dependent Rac activation is critically involved in mediating two endothelial cell events essential for angiogenesis: migration and proliferation

PKB/Akt

The nonreceptor serine/threonine kinase PKB/Akt is activated by many growth factor and adhesion receptors, including integrins, in a PI-3K-dependent manner. PKB/Akt plays a central role in the regulation of fundamental cellular processes such as glucose metabolism, transcription, cell proliferation, migration and survival [17, 107, 108]. Among the three known PKB/Akt isoforms, PKBα/Akt1 is the most ubiquitous one and is highly expressed in endothelial cells. PKB/Akt activation requires translocation to the cell membrane through binding of its pleckstrin homology domain to membrane phosphatidylinositol 3 phosphates and phosphorylation at two sites, ^{308}T and ^{473}S [109]. Many growth factors and soluble ligands, such as VEGF [110], insulin [111] and angiopoietin-1 [112] can activate PKB/Akt, but only under conditions of integrin-dependent adhesion [113]. Furthermore, integrin ligation directly activates PKB/Akt through multiple pathways, including FAK and ILK [114, 115]. PKB/Akt is a key mediator of integrin-dependent endothelial cell survival, proliferation and migration. The mechanisms by which PKB/Akt promotes these three effects are not yet fully understood. The anti-apoptotic activity involves inactivation of Bad, Bax and caspase 9, suppression of Forkhead (FRKH) transcriptional activity [116], stimulation of nitric oxide (NO) production [117, 118] and suppression of p38 signaling [119]. Additional mechanisms, however, are likely to be involved. Regulation of cell migration appears to implicate cross-talk with Rho-family proteins. In migrating cells, PKB/Akt localizes to the leading edge [120], a site of high Rac activity [121]. Recent reports have demonstrated that PKB/Akt induces Rac activation in endothelial cells [122], while others have shown that Rac and Cdc42 are situated upstream of PKB/Akt and promote its activation during cell migration [123, 124]. More recently, a direct effect of PKB/Akt on PAK activity was reported in *Dictostelium discoideum* cells [125], suggesting the possibility that PKB/Akt may regulate actin dynamics through direct phosphorylation of PAK. Endothelial cell migration induced by VEGF/VEGF-R2 stimulation involves integrin activation via a PI-3K and PKB/Akt-dependent signaling pathway, although the precise mechanism was not elucidated [126]. The regulation of the cell cycle by PKB/Akt appears to involve regulation of FRKH/E2F, MDM2 and p21 activities [127–131].

Membrane rafts and integrin signaling

Lipids in the plasma membrane are organized in distinct domains depending on their structure. Sphingolipids, characterized by long and highly saturated acyl chains, can pack tightly with cholesterol and form assemblies in a liquid-ordered phase, called membrane rafts. Rafts are surrounded by phospholipid-rich regions, which are in a more fluid, liquid-disordered state, because the kinked, unsaturated acyl chains of phospholipids can only pack

loosely. Due to their biophysical properties, rafts are insoluble in nonionic detergents at 4 °C and can be isolated from plasma membranes as detergent-insoluble glycolipid-enriched membranes (DIGs), also referred to as detergent-resistant membranes (DRMs) [132]. Several proteins can be found in rafts where they specifically interact, thus suggesting that rafts may function as platforms for the assembly of signaling complexes and play an important role in signaling regulation. Proteins can be directly targeted to rafts by posttranslational modifications, such as glycosylphosphatidylinositol (GPI) anchors, or acylated chains (e. g. miristoylation and palmitoylation as in the case of some Src-family kinases), or they can be recruited through the establishment of protein-protein interactions in response to a stimulus. Ligand binding to some transmembrane receptors leads to their translocation into rafts or to the stabilization of receptor dimers in these domains, and to the consequent activation of a signaling cascade(s) (see fig. 4). It is possible that rafts with different protein composition are present in the membrane, and that activated receptors within one type of rafts recruit proteins in other rafts, thus promoting raft clustering and interaction of proteins that otherwise cannot associate because of localization into different membrane domains [133, 134].

Several reports have shown that some integrins are in part localized to lipid rafts where they activate specific signaling pathways. With the exception of the integrin $\alpha 6\beta 4$ that can be directly targeted to rafts by palmitoylation of the $\beta 4$ subunit [135], translocation to rafts of other integrins that are not acylated is probably dependent on their interaction with proteins having higher affinity for rafts. In T cells, adhesion mediated by the integrin $\alpha L\beta 2$ requires translocation of the activated integrin into rafts. Activated $\alpha L\beta 2$ induces the release of integrin $\alpha 4\beta 1$ from the cytoskeleton and its relocalization into rafts. Cholesterol depletion, and hence raft disruption, by methyl-β-cyclodextrin prevents both $\alpha L\beta 2$- and $\alpha 4\beta 1$-dependent adhesion, indicating the importance of intact and functional lipid microdomains for integrin-mediated adhesion in T cells [136].

Complexes of integrins with some membrane glycoproteins have also been localized to rafts. Examples are the association of $\alpha V\beta 3$ with CD47 and of $\alpha 3\beta 1$ and $\alpha 6\beta 1$ with CD36. The latter complexes were found in melanoma cells, and their activation was shown to stimulate migration through not yet identified signaling pathway(s) [137]. Binding of thrombospondins to CD47 modulates the function of $\alpha V\beta 3$ in melanoma and endothelial cells, stimulating cell spreading or migration. The complex between $\alpha V\beta 3$ and the membrane pentaspanin protein CD47 seems to mimic classic sevenspanin receptors for its signaling properties. Upon ligation, it activates heterotrimeric G proteins by recruiting the Gαi subunit and inducing a decrease in intracellular cAMP. The ternary complex $\alpha V\beta 3$-CD47-Gαi can be isolated from rafts, and its integrity requires the presence of cholesterol [138, 139].

There is evidence that complexes of integrin $\alpha 3\beta 1$ with the tetraspanin proteins CD9, CD63 and CD81 can exist

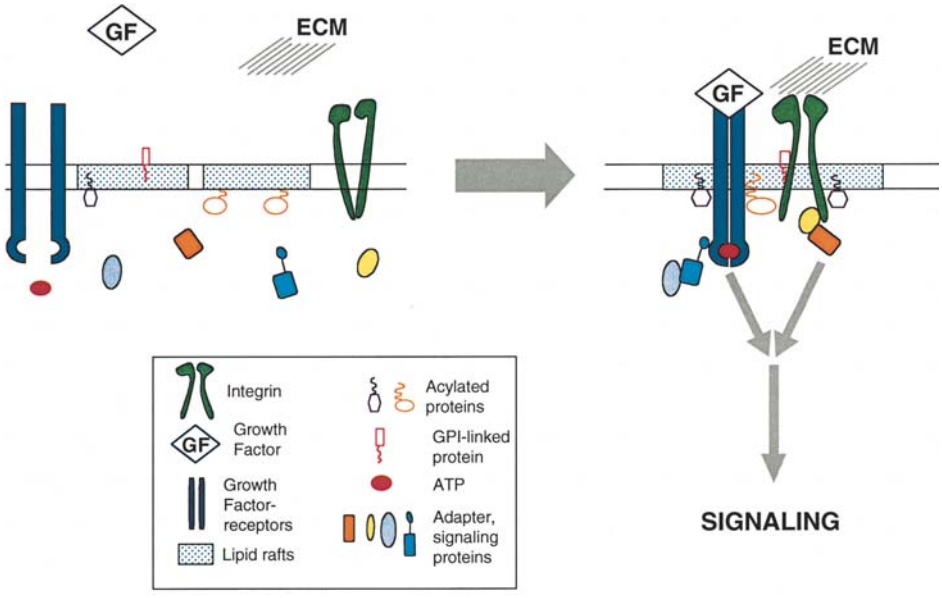

Figure 4. Model of signaling activation in membrane lipid rafts. Some proteins are directly targeted to rafts by posttranslational modifications (acylation, GPI anchors); rafts with different lipid and protein composition may exist within the membrane. Upon ligand binding and activation, some GFRs and some integrins are recruited into lipid rafts and associate with raft-resident signaling proteins. These interactions lead to the activation of signaling pathways by recruitment of cytoplasmic proteins, which thus become part of the raft-associated protein complexes.

in raftlike microdomains, but these complexes have peculiar properties that distinguish them from other raft-associated integrin complexes, and they also exist outside membrane rafts. In fact, their extraction requires detergent-free conditions or nonionic detergents other than TritonX-100 which destabilizes the association, their integrity is independent of cholesterol, and the raft-like microdomains where they are localized do not contain caveolin-1 or GPI-linked proteins [140]. These observations support the hypothesis that different types of lipid microdomains, with different protein and probably lipid composition, exist in the cell membrane, and their interactions and reorganization can participate in the regulation of signaling.

Integrin-dependent activation of extracellular regulated kinase (ERK) seems also to require integrin signaling from lipid microdomains. It was shown that integrins can form a complex with caveolin-1 and doubly acylated and raft-localized Fyn and Yes, and activate ERK through Fyn/Yes-dependent tyrosine-phosphorylation of Shc [76]. Another report showed that the GPI-linked urokinase receptor uPAR associates to β1 integrins in a caveolin-dependent manner and regulates cell adhesion and ERK activation by integrins [141]. Furthermore, it was recently shown that adhesion to fibronectin of 293T cells stimulates translocation into rafts of β1 integrins, FAK and the phosphatase Shp-2. Shp-2 activity in rafts seems to be responsible for ERK activation by promoting specific FAK phosphorylation at ^{397}Y, which does not require Src-family kinase activity. Moreover, Shp-2 signaling from rafts regulates Rho activity and thus cytoskeleton remodeling and cell spreading, probably facilitating assembly of integrin clusters that promotes FAK transphosphorylation [142].

Since changes in lipid and protein composition and structure of rafts regulate signal transduction and cell behavior, it is conceivable that abnormal alterations of membrane microdomains contribute to the development of certain diseases. In the case of endothelial cells, it is not known how raft dynamics and integrin signaling from within and outside rafts are regulated in quiescent cells and during physiological and pathological angiogenesis.

Integrins in vascular development and angiogenesis

Endothelial cells have been reported to express at least 11 different integrins: $\alpha1\beta1$, $\alpha2\beta1$, $\alpha3\beta1$, $\alpha5\beta1$, $\alpha6\beta1$, $\alpha8\beta1$, $\alpha9\beta1$, $\alpha V\beta1$, $\alpha V\beta3$, $\alpha6\beta4$ and $\alpha V\beta5$ [143–149]. Most of these integrins are receptors for collagens and laminins (see table 1), thus consistent with a role in mediating endothelial cell interaction with the basal membrane of mature vessels. Some integrins (i.e. $\alpha5\beta1$, $\alpha V\beta3$ and $\alpha V\beta5$) bind to fibronectin, vitronectin and other ECM proteins absent from the basal membrane of mature vessels but present at sites of angiogenesis.

Experimental evidence that integrins are required for embryonic vascular development and postnatal angiogenesis came from mouse experiments in which integrin genes were disrupted in embryonic stem cells (genetic approach), and from experiments based on the use of integrin antagonists (pharmacological approach) [150, 151]. Integrin $\alpha5\beta1$-deficient mice die early during embryonic development due to defects in the extra embryonic and embryonic vasculature [152]. Embryos lacking $\alpha5\beta1$ integrin have greatly distended blood vessels in the vitelline yolk sac and in the embryo itself. This defective vascular phenotype is correlated with a decreased depo-

Table 1. Integrins expressed on endothelial cells and their extracellular matrix ligands.

Integrin	Major ECM protein ligands	Integrin changes during AG	Ligand availability during AG
$\alpha1\beta1$	CO, LM	$\Leftrightarrow$	$\Downarrow$
$\alpha2\beta1$	CO, LM, VN, TN-C, TSP	$\Leftrightarrow$	$\Downarrow$ or $\uparrow$
$\alpha3\beta1$	CO, LM, FN, TSP, EL	$\Leftrightarrow$	$\Downarrow$ or $\uparrow$
$\alpha5\beta1$	FN, TSP	$\uparrow\uparrow$	$\uparrow$
$\alpha6\beta1$	LM	$\Leftrightarrow$	?
$\alpha6\beta4$	LM	?	$\Downarrow$
$\alpha8\beta1$	FN, VN, TN-C	?	$\uparrow\uparrow$
$\alpha9\beta1$ *	TN-C, OPN, CO	?	$\uparrow$
$\alpha V\beta1$	FN, VN	?	$\uparrow\uparrow$
$\alpha V\beta3$	FN, VN, FB, TSP, TN-C, vWF, dCO, OP, MMP-2, Del1	$\uparrow\uparrow$ (de novo expression)	$\uparrow\uparrow$
$\alpha V\beta5$	FN, VN	$\Leftrightarrow$	$\uparrow\uparrow$

Endothelial cells express at least 11 different integrins. Integrin $\alpha5\beta1$ and $\alpha V\beta3$ are strongly upregulated during angiogenesis (AG). The composition of the ECM changes dramatically during angiogenesis due to proteolytic degradation and de novo protein deposition/expression. $\Leftrightarrow$, unchanged; $\uparrow\uparrow$, increased; $\Downarrow$, decreased; ?, not known. * $\alpha9\beta1$ integrin is expressed in lymphatic endothelial cells but not in vascular endothelial cells. Abbreviations: CO, collagen; dCO, denatured collagen; Del1, developmental endothelial locus-1; EL, elastin; FB, fibrinogen; FN, fibronectin; LM, laminin; MMP, matrix metalloproteinase; OPN, osteopontin; TN-C, tenascin-C; TSP, thrombospondin; VN, vitronectin; vWF; von Willenbrand factor. The list of ligands is not exhaustive.

sition of fibronectin, the main ligand of $\alpha 5$ integrin, in the endothelial basement membranes [153]. Moreover, in embryonic bodies formed from embryonic stem cells lacking $\alpha 5$ integrin, a significant reduction in early capillary plexus formation and maturation was observed [153]. These findings demonstrate that successful early vasculogenesis and angiogenesis require $\alpha 5\beta 1$-fibronectin interactions. $\alpha 4\beta 1$-deficient mice die early during embryonic development due to placental defects, including abnormal vessels, or late during development due to defects in the epicardium and coronary vessels, but have no angiogenic defect in other tissues [154]. αV-deficient mice develop intestinal, placental and cerebral malformations leading to perinatal and early postnatal lethality [155]. Detailed analysis of the brain of αV-deficient mice revealed normal association between endothelial cells and pericytes but defective interaction between cerebral microvessels and the surrounding brain parenchyma [156]. $\beta 8$-deficient mice die at mid-gestation or shortly after birth due to extensive hemorrhages associated with abnormal vascular morphogenesis and distended and leaky capillary vessels in the yolk sac, placenta and brain [157]. Consistent with a role for αV integrins in mediating the interaction between microvessels and the surrounding parenchyma, the $\beta 8$ transcript was found localized in endodermal cells surrounding the endothelium in the yolk sac [157]. Inhibition of $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrin with function-blocking antibodies suppress endothelial cell attachment and migration on collagen matrices in vitro and VEGF-induced angiogenesis in vivo [158, 159]. Antagonists of integrins $\alpha V\beta 3/\alpha V\beta 5$ suppress tumor-, VEGF- and hypoxia-induced angiogenesis by selectively inducing apoptosis of angiogenic endothelial cells [160]. Strikingly, $\alpha V\beta 3$ and $\alpha V\beta 5$ were reported to promote angiogenesis in association with distinct angiogenic factors. In the cornea and CAM models, angiogenesis induced by FGF-2 or TNF depended on $\alpha V\beta 3$, while angiogenesis initiated by VEGF or TGF-α depended on $\alpha V\beta 5$ [161]. The exact physiological relevance of these distinct roles of $\alpha V\beta 3$ and $\alpha V\beta 5$ in growth-factor-induced angiogenesis remains unclear, but may reflect more complex differential regulation and signaling function of these two integrins [162, 163].

The integrin $\alpha 1$ and $\alpha 9$ subunits were recently reported to be preferentially expressed in lymphatic endothelial cells, compared with blood vascular endothelial cells [149]. Integrin $\alpha 9\beta 1$ is a receptor for the ECM proteins laminin, tenascin-C, osteopontin and collagen. Mice lacking $\alpha 9\beta 1$ integrin develop edema and lymphocytic infiltrates in the chest wall around putative lymphatic vessels and die perinatally due to the accumulation of lipid-rich lymph in the pleural cavity [164]. This phenotype is consistent with a role for $\alpha 9\beta 1$ in the normal development or homeostasis of lymphatic vessels, including the thoracic duct. The role of $\alpha 9\beta 1$ and $\alpha 1\beta 1$ in postnatal lymphangiogenesis remains to be addressed.

Vascular integrins as survival factors

Integrins are emerging as essential determinants of endothelial cell survival. Loss of endothelial cell adhesion and impaired spreading results in rapid onset of apoptosis [165–167], a phenomenon also referred to as anoikis [168]. Treatment of tumor-bearing mice with integrin antagonists [169], or expression of transdominant-negative integrin mutants in quiescent rat carotid artery endothelial cells causes endothelial cell death [G. Vassalli et al., unpublished].

While it is clear that adhesion promotes survival, and detached cells rapidly die, the mechanism by which detached cells die is still a matter of debate [170]. There is evidence that nonattached cells die because they lack integrin-mediated survival signals. These include increased expression of Bcl-2 [171–173], phosphorylation of the Rb tumor suppressor protein [174], inhibition of p53 function [172], activation of MEKK-1/ERK [175, 176], PKB/Akt [177, 178] and NF-κB [92, 179]. On the other hand, there is evidence that detachment may actively induce apoptosis. For example, matrix detachment induces rapid JNK activation [171]. Since JNK-1 and -2 function are essential for stress-induced apoptosis in fibroblasts, [180], it is possible that detachment-induced JNK activation promotes anoikis. Caspase 8 activation is observed in suspended cells soon after detachment [181, 182], and unligated integrins were recently shown to directly associate with and activate pro-caspase 8 [183]. The fact that cell detachment promotes dissolution of focal adhesions and of the normal cytoskeletal architecture suggests a direct positive effect of the disrupted cytoskeleton on the induction of anoikis. Indeed, regulators of apoptosis such as Bim and Bmf associate with the cytoskeleton, and cytoskeletal breakdown promotes their release and induction of apoptosis [184, 185].

The role of death receptors in anoikis remains controversial. Loss of integrin ligation was reported to cause an increased production of Fas-ligand and a diminished expression of c-Flip (an endogenous inhibitor of caspase 8), resulting in Fas-mediated death [186]. Also, dominant-negative Fas-associated death domain (FADD) protein was shown to suppress anoikis [181, 182]. Nevertheless, definitive evidence for an involvement of cell surface death receptors and assembly of a death-inducing signaling complex in anoikis is still missing.

The calcium-dependent serine/threonine kinase death-associated protein kinase (DAPK) was recently shown to promote apoptosis by suppressing integrin-mediated cell adhesion and signal transduction and by upregulating p53 expression. Signaling from FAK blocked the ability of

DAPK to upregulate p53 expression and to cause apoptosis. DAPK activation may play a role in mediating anoikis, as anoikis-sensitive cells were killed by DAPK activation, while anoikis-resistant cells were not [187].
A recent report demonstrated that integrin $\alpha5\beta1$ promotes endothelial cell survival during angiogenesis by suppressing caspase-induced apoptosis in a PKA-dependent manner. $\alpha5\beta1$ ligation suppressed activation of PKA and caspase 8, while anti-$\alpha5\beta1$ inhibitory antibodies promoted sustained PKA activation, resulting in caspase 8-dependent apoptosis [188]. Furthermore, parathyroid-related peptide, a naturally occurring angiogenesis inhibitor, suppressed endothelial cell migration and angiogenesis and promotes endothelial cell apoptosis by activating PKA [189]. Rapid and transient increase in PKA activity, however, is essential for $\alpha V\beta3$ integrin-mediated endothelial cell adhesion and spreading [104]. Thus, it appears that PKA activation may have a dual function: rapid and transient PKA activation during cell adhesion promotes cell adhesion, spreading and migration, while sustained PKA activation in adherent cells leads to suppression of migration, caspase activation and apoptosis. Take together, these studies suggest that PKA, or PKA-mediated signaling events, may represent useful targets to inhibit angiogenesis.

Vascular integrin $\alpha V\beta3$: a positive or negative regulator of angiogenesis?

Several studies indicate that integrin $\alpha V\beta3$ is an important modulator of postnatal angiogenesis [190]. $\alpha V\beta3$ is highly expressed in angiogenic endothelial cells in wound granulation tissue and in malignant tumors [191–195]. $\alpha V\beta3$ ligation to ECM proteins mediates signaling events consistent with stimulation of angiogenesis. First, it promotes endothelial cell proliferation by inducing sustained MAPK activity [78], suppressing p53 activity and reducing $p21^{WAF}$ expression [172, 196]. Second, it enhances cell survival through the activation of NF-κB [92, 93] and the increase in the Bcl-2/Bax ratio [172] and suppression of caspase-8 activation [183]. Third, it stimulates endothelial cell motility [197] and localizes MMP-2 at sites of cell invasion [198]. Fourth, VEGF activates $\alpha V\beta3$, resulting in enhanced integrin ligand binding, cell adhesion and migration [126]. In turn, ligated $\alpha V\beta3$ associates with VEGF-R2, resulting in enhanced VEGF-R2 signaling and endothelial cell proliferation [199]. Extracellular antagonists of $\alpha V\beta3$ integrin are potent inhibitors of angiogenesis. An $\alpha V\beta3$ function-blocking monoclonal antibody (LM609) [200] and an $\alpha V\beta3/\alpha V\beta5$ antagonistic cyclic peptide (EMD121974) [201] suppress cornea vascularization [161] and hypoxia-induced retinal neovascularization in the mouse [202], tumor angiogenesis in the chick CAM model [169, 192] and in several mouse tumor models [203, 204]. Importantly, preexisting vessels are not affected by $\alpha V\beta3$ antagonists. Also, the use of small molecular antagonists that inhibit MMP-2 binding to $\alpha V\beta3$ was recently reported to suppress angiogenesis [205]. The observations that $\alpha V\beta3$ is de novo expressed in angiogenic endothelial cells, that $\alpha V\beta3$ ligation induces cellular effects consistent with an 'angiogenic' endothelial cell phenotype and that $\alpha V\beta3$ antagonists efficiently suppress angiogenesis have led to the notion that integrin $\alpha V\beta3$ is a critical positive mediator of angiogenesis. Based on these preclinical data, EMD121974 (Cilengitide) and humanized LM609 (Vitaxin) are currently being tested in phase II clinical trials for their therapeutic efficacy in human cancer. Phase I trials demonstrated that these drugs are safe and well tolerated [206].
Recent observations obtained from the study of genetically modified mice lacking one or more of the αV-containing integrins, however, raised some serious questions on the role of αV integrins in angiogenesis and on the interpretation of the experimental results obtained with the pharmacological antagonists. Deletion of the αV gene by homologous recombination causes loss of five αV-containing integrins: $\alpha V\beta1$, $\alpha V\beta3$, $\alpha V\beta5$, $\alpha V\beta6$, $\alpha V\beta8$. The observation that mice lacking the αV gene have extensive vasculogenesis and angiogenesis was, therefore, a great surprise [155]. Although αV-deficient mice die peri-/postnatally due to bleeding in the brain and intestine, suggesting nevertheless some vascular-related function of αV integrins, it is clear that αV integrins are largely dispensable for developmental angiogenesis. Moreover, mice lacking $\alpha V\beta3$, $\alpha V\beta5$ or both integrins are viable, have no detectable defects in developmental angiogenesis, display enhanced postnatal angiogenesis in response to hypoxia, VEGF and tumor cells, and have an accelerated tumor growth [207]. The dispensable role of $\alpha V\beta3$ in developmental angiogenesis is consistent with the observation that mutations in the $\beta3$ gene leading to deficient $\alpha V\beta3$ and $\alpha IIb\beta3$ integrin expression or function is not embryonic lethal in mouse or human. Humans with a nonfunctional $\beta3$ subunit suffer of severe bleeding due to impaired platelet aggregation, a condition known as Glanzmanns's thrombasthenia, but have normal angiogenesis [208, 209]. Intriguingly, the divergent effects on angiogenesis observed following pharmacological vs. genetic inactivation of $\alpha V\beta3$, are not observed in other situations involving $\alpha V\beta3$ function. For example, $\alpha V\beta3$-dependent osteoclast adhesion and bone resorption are impaired by both $\beta3$ gene deletion and $\alpha V\beta3$ antagonists, resulting in an osteopetrotic phenotype [210, 211].
While these discordant results (i.e. normal developmental angiogenesis and excessive tumor angiogenesis in $\alpha V\beta3$-deficient mice and suppression of angiogenesis in wild-type mice by pharmacological $\alpha V\beta3$ antagonists) are now widely accepted, we still lack a comprehensive understanding of the mechanisms involved. At least three

possible mechanisms may be considered. A first putative explanation is functional compensation. Although no apparent upregulation (i.e. genetic compensation) of other integrins is observed in β3-deficient mice [207], lack of αVβ3 may be functionally compensated by another vascular integrin normally expressed by endothelial cells. Integrin α5β1 would be an attractive candidate since it is essential for vascular development [152, 153], promotes cell survival [178], induces development of an angiogenic endothelial cell phenotype in vitro [94] and α5β1 antagonists inhibit angiogenesis in vivo [212]. In αVβ3-expressing angiogenic endothelial cells, the function of α5β1 and α2β1 integrins may be suppressed by active αVβ3 through a transdominant negative effect, as it has been demonstrated for αIIbβ3 [213]. The mitogenic activity of αVβ3 may be compensated by other nonintegrin receptors. Since αVβ3 ligation facilitates VEGF-R2 phosphorylation and signaling [199], the enhanced VEGF-R2 expression in αVβ3-deficient mice may be a compensatory mechanism to rescue αVβ3-negative endothelial cells during development [207]. Enhanced VEGF-R2 expression during adult life renders endothelial cells more sensitive to VEGF, resulting in increased endothelial cell proliferation and angiogenesis [207].

A second putative mechanism is integrin-mediated death [214]. This has been recently proposed based on the observation that expression of unligated integrin heterodimers or isolated integrin β cytoplasmic subunits causes apoptosis through the recruitment and activation of caspase 8 [183]. This model proposes that the ligation state of αVβ3 determines suppression or induction of apoptosis. Expression of αVβ3 integrin in the absence of a ligand would activate caspase 8 clustering and activation, leading to caspase 3 activation and induction of apoptosis. In contrast, αVβ3 ligation prevents spontaneous caspase 8 activation and induction of apoptosis, resulting in enhanced cell survival. In the absence of αVβ3 expression, cells would become insensitive to integrin-mediated death, resulting in enhanced survival and angiogenesis, as observed in the αVβ3-deficient mice [207].

A third recently proposed mechanism suggests that αVβ3 may be a negative regulator of angiogenesis [215]. This concept is supported by the observation that some natural ligands of αVβ3, such as thrombospondin-1 and -2 [216, 217], the MMP-2 proteolytic product PEX [218–220] and tumstatin, a proteolytic fragment of collagen IV α3 chain [221, 222], are known inhibitors of angiogenesis. In addition, RGD-based small molecular inhibitors and some function-blocking antibodies that bind to αVβ3 can activate αVβ3 and may therefore stimulate intracellular signaling, while at the same time inhibiting αVβ3-mediated cell adhesion. The 'negative regulator' hypothesis, however, does not account for the large body of evidence demonstrating that αVβ3 ligation induces endothelial cell proliferation, migration and survival as expected for angiogenic endothelial cells [22, 92, 172, 183, 195]. It is, however, possible that αVβ3 have both positive and negative function depending on the substrate and on the context (e.g. presence of particular cytokines, proliferation or differentiation state of the cell) [70]. Taken together, it is reasonable to assume that we are still missing a critical link in our understanding of the role of αVβ3 and angiogenesis.

Integrins, COX-2 and tumor angiogenesis

Nonsteroidal antiinflammatory drugs (NSAIDs) exert antitumor effects on premalignant lesions and established experimental tumors. These effects are largely dependent on the inhibition of cyclooxygenase-2 (COX-2), a key enzyme in the synthesis of prostaglandins and thromboxans, highly expressed in inflammation and cancer [223, 224]. Recent evidence indicates that the antitumor activity of NSAIDs and selective COX-2 inhibitors is associated with suppression of tumor angiogenesis. Deletion of the COX-2 gene in mice suppressed VEGF production by fibroblasts, and treatment of wild-type fibroblasts with a selective COX-2 inhibitor reduced VEGF production [225]. Overexpression of COX-2 in cancer cells induced expression of VEGF and other angiogenic factors [226]. COX-2 also regulates VEGF-R2 function in endothelial cells. NSAIDs block VEGF-R2-dependent increased vascular permeability [227] and MAPK activation in response to VEGF [228]. Endothelial cell COX-2 has been recently reported to be a positive regulator of vascular integrin αVβ3 function [102]. Inhibition of COX-2 activity in endothelial cells by NSAIDs results in a diminished αVβ3-dependent endothelial cell spreading and migration in vitro, and FGF-2-induced angiogenesis in vivo. This effect is due to the inhibition of αVβ3-dependent activation of Cdc42 and Rac, two members of the Rho family of GTPases that regulate cytoskeletal organization and cell migration. Besides promoting Rac activation and cell spreading, the COX-2 metabolite PGE_2 also accelerates αVβ3-mediated endothelial cell adhesion by controlling the cyclic AMP (cAMP) level upon αVβ3 engagement. PGE_2 exerts these effects through activation of the cAMP-dependent protein kinase PKA and induces αVβ3-dependent spreading via cAMP- and PKA-dependent Rac activation [104]. These results demonstrate that inhibition of αVβ3-mediated Rac activation is an important mechanism by which NSAIDs suppress angiogenesis and establish a novel functional link between inflammation and angiogenesis. Intriguingly, overexpression of COX-2 in the skin and in the breast was recently reported to promote tumorigenesis and tumor progression, and this effect was associated with enhanced local PGE_2 production and angiogenesis [229, 230].

Integrin ligands and their modification during angiogenesis

Quiescent endothelial cells adhere to the vessel wall via the subendothelial basal membrane, consisting mostly of laminins and collagens. During angiogenesis the interaction between endothelial cells and the extracellular matrix changes dramatically: matrix proteinases of the MMP family cleave existing ECM proteins, and new ECM proteins are deposited. These changes in the ECM generate new substrate adhesion sites promoting endothelial cell migration. For example, proteolytic cleavage of collagen IV eliminates binding of integrin $\alpha1\beta1$ and reveals a cryptic site recognized by integrins $\alpha V\beta3$ [231], while MMP-2-mediated cleavage of collagen I results in the exposure of an RGD-binding site for integrin $\alpha V\beta3$ [232]. Furthermore, proteolytic cleavage of certain ECM proteins releases soluble fragments acting as positive or negative regulators of cell migration or angiogenesis. For example, MMP-2-mediated cleavage of laminin 5 releases a fragment that increases $\alpha3\beta1$-dependent cell migration [233, 234]. Endostatin and tumstatin, two endogenous inhibitors of angiogenesis reported to interact with vascular integrins, including $\alpha V\beta3$, are generated by the proteolytic processing of collagen XVIII and collagen IV, respectively [221, 222, 235, 236]. In addition to the proteolytic processing of the existing subendothelial and perivascular ECM, angiogenesis requires de novo deposition of ECM proteins. This occurs through two distinct mechanisms. First, adhesion-promoting serum proteins, in particular fibrinogen, fibronectin and vitronectin, extravasate into the perivascular space following increased vascular permeability in response to VEGF [237]. Serum proteins accumulating in the perivascular ECM and proteolitically modified ECM proteins provide a provisional matrix that acts as a substrate for 'angiogenic' integrins (e.g. $\alpha V\beta3$ and $\alpha5\beta1$). The importance of the extravasion of serum proteins in early phases of angiogenesis has been recently challenged by the observation that inhibition of Src-family kinases by retroviral expression of a dominant-negative construct impaired VEGF-induced vascular leakage but not angiogenesis [238]. Second, endothelial cells, infiltrating leukocytes and activated tissue fibroblasts produce and deposit ECM proteins that are usually absent, or expressed at very low levels, in normal vessels and tissue. These include ED-A$^+$ and ED-B$^+$ fibronectin ('oncofetal fibronectin') [239], tenascin-C (TN-C) [240], thrombospondin-1 and -2 [216] and SPARC [241]. TN-C, thrombospondins and SPARC may have a dual function in angiogenesis: they may destabilize established adhesion at early phases of angiogenesis, while promoting adhesion and migration at later time points [241]. The contribution of ED-A+ and ED-B+ fibronectin in promoting angiogenesis remains to be demonstrated.

Inhibition of integrins and integrin-dependent signaling to suppress angiogenesis and lymphangiogenesis

It is quite clear that integrins are important mediators and regulators of angiogenesis during physiological and pathological situations, and that integrin antagonists are effective in inhibiting progression of angiogenesis-dependent diseases such as chronic inflammation, proliferative retinopathy and tumor progression. The efficacy of $\alpha V\beta3$ integrin antagonists to suppress angiogenesis in human cancer is currently being tested in clinical trials. The results of these trials will tell us whether vascular integrin $\alpha V\beta3$ is a good target and whether the currently available pharmacological $\alpha V\beta3$ antagonists are effective drugs. Recent advances in the field, however, have suggested alternative strategies to suppress tumor angiogenesis by targeting other integrins or integrin-dependent signaling pathways. We will briefly summarize here some of these findings and their implications (see fig. 5).

Alternative integrins

While $\alpha V\beta3$ has been originally considered as the relevant integrin to inhibit pathological angiogenesis, recent findings suggest that $\alpha5\beta1$ may also be a valuable target. $\alpha5\beta1$-deficient mice do not develop a vascular system, $\alpha5\beta1$-null endothelial cells are unable to form normal cordlike structure, $\alpha5\beta1$ is upregulated in angiogenesis and a blocking anti-$\alpha5$ mAb suppresses VEGF-induced angiogenesis in both chick embryo and murine models [188, 212]. $\alpha5\beta1$-blocking peptides have been reported [212, 242], but their use as anti-angiogenic agents has remained limited. The generation of potent high-affinity $\alpha5\beta1$ antagonists may be necessary before systematic testing of the role of $\alpha5\beta1$ in tumor angiogenesis can be envisaged in mice and humans. Among other possible alternative targets, $\alpha6\beta4$ and $\alpha2\beta1$ should be considered. A recent report suggests that $\alpha9\beta1$ is expressed on lymphatic but not on vascular endothelial cells. Availability of $\alpha9\beta1$ antagonists may allow one to explore whether $\alpha9\beta1$ integrin also plays a role in postnatal lymphangiogenesis, including tumor lymphangiogenesis [243, 244].

Integrins as 'vascular addressins' for drug targeting

The finding that $\alpha V\beta3$ is preferentially expressed at high levels on endothelial cells of the tumor vasculature suggested the possibility of using it as a 'vascular addressin' to selectively target drugs to tumors [194, 245]. Coupling of an αV-binding RGD motif to the anticancer drug doxorubicin resulted in enhanced drug targeting to the tumor vasculature, increased doxorubicin efficacy against human breast cancer xenografts in nude mice and reduced systemic toxicity [246]. A paramagnetic contrast agent

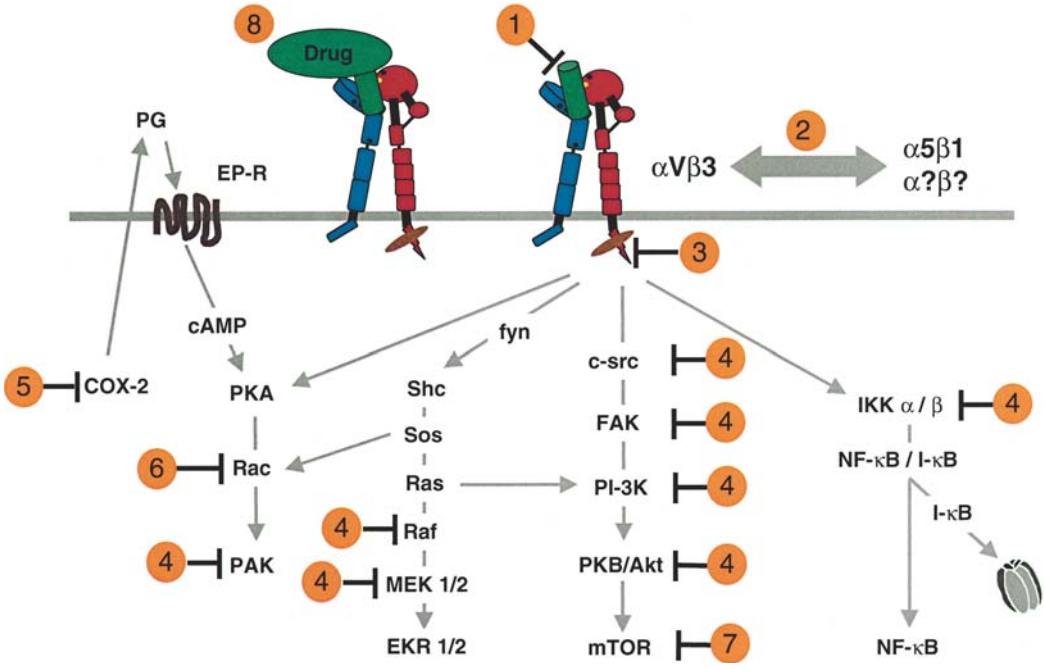

Figure 5. Targeting integrins or integrin-dependent signaling pathways to inhibit angiogenesis. (1) Inhibition of integrin ligand binding with blocking antibodies or small pocket antagonists; (2) $\alpha V\beta 3$ has been the first integrin targeted to suppress angiogenesis, but recent evidence suggests that other integrins, in particular $\alpha 5\beta 1$, should be considered as possible targets; (3) interfering with the integrin cytoplasmic domain using dominant-negative constructs completely inhibits integrin function; (4) several nonreceptor kinases downstream of integrins are candidate targets for pharmacological inhibitors or dominant-negative constructs to block angiogenesis; (5) COX-2 antagonists suppress prostaglandin production and block angiogenesis by inhibiting $\alpha V\beta 3$-mediated activation of Rac; (6) inhibition of Rac by dominant-negative constructs potently block angiogenesis. To date there are no pharmacological inhibitors of Rac; (7) the PKB/Akt target mTOR can be inhibited by rapamycin, resulting in suppression of tumor angiogenesis; (8) vascular integrin $\alpha V\beta 3$ has been used as an 'addressin' to target chemotherapeutic drugs, nanoparticles, liposomes or imaging-enhancing agents to tumor vessels.

targeted to vascular $\alpha V\beta 3$ via the LM609 monoclonal antibody was used to detect angiogenesis in vivo by magnetic resonance imaging [247]. This approach provided enhanced and detailed imaging of rabbit carcinoma, including angiogenic 'hot spots' not seen by standard magnetic resonance imaging. Recently, cationic nanoparticle coupled to a high-affinity $\alpha V\beta 3$ ligand was used to selectively target a firefly luciferase-encoding plasmid to the vasculature of established tumors [248]. Single delivery of a plasmid encoding a dominant-negative Raf molecule resulted in apoptosis of the tumor vasculature and tumor cells, and regression of primary and metastatic tumors. Since $\alpha V\beta 3$ is a coreceptor for several viruses, including adenovirus [249], rotavirus [250] and foot-and-mouth virus [251], it may be exploited for enhanced virus targeting to tumor sites [252].

Suppressing $\alpha V\beta 3$ signaling with TNF and IFNγ

Administration of high doses of tumor necrosis factor (TNF), together with gamma interferon (IFNγ) and melphalan through the isolated limb perfusion technique to patients with advanced melanoma and soft tissue sarcoma of the limbs, results in extensive tumor necrosis (80–90% complete response in melanoma and 35% complete response in soft tissue sarcoma) [253, 254]. This is the first registered anticancer treatment in which disruption of the tumor vasculature is an essential component of the therapeutic effect [255]. TNF and IFNγ synergistically inhibit cell proliferation [256] via a G1 cycle arrest associated with reduced levels of cyclin D1 and cdk2, increased expression of the cdk inhibitors $p16^{INK4a}$, $p21^{WAF}$ and $p27^{Kip1}$ and hypophosphorylation of the Rb protein [257]. Endothelial cells exposed to TNF/IFNγ selectively lose $\alpha V\beta 3$-dependent adhesion. This is due to the inhibition of $\alpha V\beta 3$ function and signaling and not to decreased cell surface expression [258]. Culture of TNF/IFNγ-treated endothelial cells on a $\alpha V\beta 3$ matrix results in an increased rate of apoptosis, while detachment and apoptosis of angiogenic endothelial cells in the tumor vasculature is observed after TNF/IFNγ administration [258]. Taken together, these results implicate changes in the function of integrin $\alpha V\beta 3$ in the effects of TNF and IFNγ on the tumor vasculature. The elucidation of the molecular mechanism by which TNF and IFNγ suppress $\alpha V\beta 3$-signaling and disrupt the tumor vasculature is an essential step in improving the clinical use of TNF and to the development of alternative drugs exploiting the mechanisms of action of TNF, but lacking its undesired effects.

Dominant-negative integrins

Interfering with the integrin subunit cytoplasmic tail perturbs integrin activity. Expression of an isolated β-subunit cytoplasmic domain suppresses integrin function and localization, cell spreading, migration and matrix assembly in fibroblasts, embryonic kidney cells and NIH 3T3 cells [259, 260]. Infection of confluent monolayers of endothelial cells with a recombinant adenovirus expressing a dominant-negative β integrin results in rapid endothelial cell detachment from the substrate [261]. Detached cells fail to readhere to matrix proteins and rapidly die by apoptosis [261]. This inhibitory effect was shown to be transdominant, since all expressed integrins were inhibited. Local delivery of an adenovirus expressing the dominant-negative construct into rat arteries results in endothelial cell rounding and detachment from the vessel wall, while cells infected with the control construct retain their flattened morphology and do not detach. DNA fragmentation (TUNEL assay) and active caspase 3 occur in dominant-negative integrin-expressing cells but not in cells expressing the control construct [G. Vassalli et al., unpublished]. This approach is very effective, but so far it does not allow selective targeting of one specific integrin (e.g. $\alpha V\beta 3$ vs. $\beta 1$ integrins). This limitation may be solved by using shorter regions of the cytoplasmic domains. Indeed, a peptide carrying the very carboxyl-terminal residues (i.e. 747–762) of the integrin $\beta 3$ cytoplasmic domain was reported to inhibit $\beta 3$-, but not $\beta 1$-mediated cell adhesion [262]. Alternatively, one may take advantage of the fact that some cytoplasmic proteins specifically bind to the cytoplasmic domain of one particular integrin (e.g. $\beta 3$-endonexin binds to the $\beta 3$ cytoplasmic domain) [263] to develop antagonistic drugs specific for $\beta 3$ integrin.

Inhibition of nonreceptor kinases

Integrins have no intrinsic kinase activity and need to recruit and activate a number of nonreceptor protein and lipid kinases to transduce signaling, including Src-family kinases (e.g. c-src, fyn, yes), FAK, PKA, Pyk-2 and integrin linked kinase (ILK) [66, 67]. Integrin-mediated adhesion and migration depend on c-src, yes and fyn signaling [264]. FAK-null fibroblasts have an increased number of focal adhesions and cannot spread and migrate properly [265, 266]. PI-3K, PKB/Akt and ILK mediate motility and survival [114, 267]. The pathways activated by these kinases may therefore represent relevant pharmacological targets downstream of integrins [268]. This concept was elegantly demonstrated by two recent reports. Tumstatin, a 28-kDa proteolytic fragment of collagen IV α chain with anti-angiogenic activities [221], was shown to suppress $\alpha V\beta 3$-dependent activation of the FAK-PI-3K-PKB/Akt-mTOR pathway, resulting in decreased protein synthesis [222]. Consistent with this report, pharmacological inhibition of mTOR with rapamycin was shown to suppress tumor angiogenesis and primary and metastatic tumor growth [269].

Inhibition of COX-2-Rac-PAK pathway

NSAIDs prevent cancer progression, at least in part, by suppressing tumor angiogenesis. Inhibition of $\alpha V\beta 3$/Rac-dependent endothelial cell migration and angiogenesis is part of the mechanism of action of NSAIDs in cancer [104, 270]. The potential therapeutic activity of NSAIDs in human cancer is currently addressed in clinical trials [224]. Furthermore, the naturally occurring angiogenesis inhibitor parathyroid hormone-related peptide inhibits endothelial cell migration and angiogenesis through inhibition of Rac activation [189]. Inhibition of Rac function in angiogenic endothelial cells should therefore be considered as an alternative strategy to suppress angiogenesis. Since Rac is expressed in virtually all cells and controls essential cellular functions, such as cell polarity, proliferation, migration and vesicular trafficking, it is likely that direct pharmacological inhibition of Rac will have severe toxic effects [271, 272]. The fact that NSAIDs prevent Rac activation induced by $\alpha V\beta 3$, but not by $\alpha 5\beta 1$, suggests the existence of a specific and NSAIDs-sensitive $\alpha V\beta 3$-dependent signaling pathway or GEFs/GAPs. The identification of this pathway may lead to the design of drugs that selectively suppress Rac activation in angiogenic endothelial cells.

Outlook

In about a decade's time, vascular integrins were recognized as critical regulators and mediators of angiogenesis, including tumor angiogenesis. Several integrin-dependent signaling pathways contributing to angiogenesis were deciphered, and integrin antagonists with anti-angiogenic activity were generated. This is a remarkable achievement for such a short time. The potential efficacy of integrin antagonists to suppress tumor angiogenesis and human cancer progression (alone or in combination with other forms of antitumor therapy) is currently being addressed in clinical trials. Despite (or because) of this rapid progress, many questions remain open, and new ones are emerging. It will be of invaluable importance to understand the exact role of integrin $\alpha V\beta 3$ in angiogenesis, to elucidate mechanisms by which integrin antagonists inhibit angiogenesis, and to obtain information on how these inhibitors can be best used in the clinics. Thus, the prospects for the study of vascular integrins and for the translation of experimental results into clinical applications promise to be even more exciting then those of the past.

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1 Folkman J. (2001) Angiogenesis-dependent diseases. Semin. Oncol. **28:** 536–542
2 Kerbel R. and Folkman J. (2002) Clinical translation of angiogenesis inhibitors. Nat. Rev. Cancer **2:** 727–739
3 Risau W. and Flamme I. (1995) Vasculogenesis. Annu. Rev. Cell. Dev. Biol. **11:** 73–91
4 Risau W. (1997) Mechanisms of angiogenesis. Nature **386:** 671–674
5 Breier G. (2000) Angiogenesis in embryonic development – a review. Placenta 21 **Suppl. A:** S11–15
6 Patan S. (2000) Vasculogenesis and angiogenesis as mechanisms of vascular network formation, growth and remodeling. J. Neurooncol. **50:** 1–15
7 Shibuya M. (2001) Structure and function of VEGF/VEGF-receptor system involved in angiogenesis. Cell Struct. Funct. **26:** 25–35
8 Powers C. J., McLeskey S. W. and Wellstein A. (2000) Fibroblast growth factors, their receptors and signaling. Endocr. Relat. Cancer **7:** 165–197
9 Jones N., Iljin K., Dumont D. J. and Alitalo K. (2001) Tie receptors: new modulators of angiogenic and lymphangiogenic responses. Nat. Rev. Mol. Cell. Biol. **2:** 257–267
10 Cheng N., Brantley D. M. and Chen J. (2002) The ephrins and Eph receptors in angiogenesis. Cytokine Growth Factor Rev. **13:** 75–85
11 Eliceiri B. P. and Cheresh D. A. (2001) Adhesion events in angiogenesis. Curr. Opin. Cell Biol. **13:** 563–568
12 Blaschuk O. W. and Rowlands T. M. (2000) Cadherins as modulators of angiogenesis and the structural integrity of blood vessels. Cancer Metastasis Rev. **19:** 1–5
13 Ruoslahti E. (1999) Fibronectin and its integrin receptors in cancer. Adv. Cancer Res. **76:** 1–20
14 Pepper M. S. (2001) Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. Arterioscler. Thromb. Vasc. Biol. **21:** 1104–1117
15 Herrera R. and Sebolt-Leopold J. S. (2002) Unraveling the complexities of the Raf/MAP kinase pathway for pharmacological intervention. Trends Mol. Med. **8:** S27–31
16 Tortora G. and Ciardiello F. (2002) Protein kinase A as target for novel integrated strategies of cancer therapy. Ann. N. Y. Acad. Sci. **968:** 139–147
17 Shiojima I. and Walsh K. (2002) Role of Akt signaling in vascular homeostasis and angiogenesis. Circ. Res. **90:** 1243–1250
18 Ridley A. J. (2001) Rho family proteins: coordinating cell responses. Trends Cell Biol. **11:** 471–477
19 Semenza G. L. (2000) HIF-1: using two hands to flip the angiogenic switch. Cancer Metastasis Rev. **19:** 59–65
20 Benezra R., Rafii S. and Lyden D. (2001) The Id proteins and angiogenesis. Oncogene **20:** 8334–8341
21 De Martin R., Hoeth M., Hofer-Warbinek R. and Schmid J. A. (2000) The transcription factor NF-kappa B and the regulation of vascular cell function. Arterioscler. Thromb. Vasc. Biol. **20:** E83–88
22 Boudreau N., Andrews C., Srebrow A., Ravanpay A. and Cheresh D. A. (1997) Induction of the angiogenic phenotype by Hox D3. J. Cell Biol. **139:** 257–264
23 Myers C., Charboneau A. and Boudreau N. (2000) Homeobox B3 promotes capillary morphogenesis and angiogenesis. J. Cell Biol. **148:** 343–351
24 Yancopoulos G. D., Davis S., Gale N. W., Rudge J. S., Wiegand S. J. and Holash J. (2000) Vascular-specific growth factors and blood vessel formation. Nature **407:** 242–248
25 Allt G. and Lawrenson J. G. (2001) Pericytes: cell biology and pathology. Cells Tissues Organs **169:** 1–11
26 Jung Y. D., Liu W., Reinmuth N., Ahmad S. A., Fan F., Gallick G. E. et al. (2001) Vascular endothelial growth factor is upregulated by interleukin-1 beta in human vascular smooth muscle cells via the P38 mitogen-activated protein kinase pathway. Angiogenesis **4:** 155–162
27 Thurston G. (2002) Complementary actions of VEGF and angiopoietin-1 on blood vessel growth and leakage. J. Anat. **200:** 575–580
28 Holash J., Wiegand S. J. and Yancopoulos G. D. (1999) New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. Oncogene **18:** 5356–5362
29 Holash J., Maisonpierre P. C., Compton D., Boland P., Alexander C.R., Zagzag D. et al. (1999) Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science **284:** 1994–1998
30 Chang Y. S., di Tomaso E., McDonald D. M., Jones R., Jain R. K. and Munn L. L. (2000) Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. Proc. Natl. Acad. Sci. USA **97:** 14608–14613
31 Morikawa S., Baluk P., Kaidoh T., Haskell A., Jain R. K. and McDonald D. M. (2002) Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. Am. J. Pathol. **160:** 985–1000
32 Eberhard A., Kahlert S., Goede V., Hemmerlein B., Plate K. H. and Augustin H. G. (2000) Heterogeneity of angiogenesis and blood vessel maturation in human tumors: implications for antiangiogenic tumor therapies. Cancer Res. **60:** 1388–1393
33 Asahara T., Murohara T., Sullivan A., Silver M., van der Zee R., Li T. et al. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science **275:** 964–967
34 Asahara T., Masuda H., Takahashi T., Kalka C., Pastore C., Silver M. et al. (1999) Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ. Res. **85:** 221–228
35 Isner J. M., Kalka C., Kawamoto A. and Asahara T. (2001) Bone marrow as a source of endothelial cells for natural and iatrogenic vascular repair. Ann. N. Y. Acad. Sci. **953:** 75–84
36 Luttun A., Carmeliet G. and Carmeliet P. (2002) Vascular progenitors: from biology to treatment. Trends Cardiovasc. Med. **12:** 88–96
37 Lyden D., Hattori K., Dias S., Costa C., Blaikie P., Butros L. et al. (2001) Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat. Med. **7:** 1194–1201
38 Alitalo K. and Carmeliet P. (2002) Molecular mechanisms of lymphangiogenesis in health and disease. Cancer Cell **1:** 219–227
39 Stacker S. A., Achen M. G., Jussila L., Baldwin M. E. and Alitalo K. (2002) Lymphangiogenesis and cancer metastasis. Nat. Rev. Cancer **2:** 573–583
40 Saaristo A., Veikkola T., Tammela T., Enholm B., Karkkainen M. J., Pajusola K. et al. (2002) Lymphangiogenic gene therapy with minimal blood vascular side effects. J. Exp. Med. **196:** 719–730
41 He Y., Kozaki K., Karpanen T., Koshikawa K., Yla-Herttuala S., Takahashi T. et al. (2002) Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. J. Natl. Cancer Inst. **94:** 819–825
42 Hynes R. O. (1992) Integrins: versatility, modulation and signaling in cell adhesion. Cell **69:** 11–25

43 Hynes R. O. (1999) Cell adhesion: old and new questions. Trends Cell Biol. **9:** M33–37
44 Giancotti F. G. and Ruoslahti E. (1990) Elevated levels of the alpha 5 beta 1 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. Cell **60:** 849–859
45 Thomas G. J., Lewis M. P., Hart I. R., Marshall J. F. and Speight P. M. (2001) AlphaVbeta6 integrin promotes invasion of squamous carcinoma cells through up-regulation of matrix metalloproteinase-9. Int. J. Cancer **92:** 641–650
46 McEver R. P. (2001) Adhesive interactions of leukocytes, platelets, and the vessel wall during hemostasis and inflammation. Thromb. Haemost. **86:** 746–756
47 Woodside D. G., Liu S. and Ginsberg M. H. (2001) Integrin activation. Thromb. Haemost. **86:** 316–323
48 Lee J. O., Bankston L. A., Arnaout M. A. and Liddington R. C. (1995) Two conformations of the integrin A-domain (I-domain): a pathway for activation? Structure **3:** 1333–1340
49 Qu A. and Leahy D. J. (1996) The role of the divalent cation in the structure of the I domain from the CD11a/CD18 integrin. Structure **4:** 931–942
50 Qu A. and Leahy D. J. (1995) Crystal structure of the I-domain from the CD11a/CD18 (LFA-1, alpha L beta 2) integrin. Proc. Natl. Acad. Sci. USA **92:** 10277–10281
51 Emsley J., King S. L., Bergelson J. M. and Liddington R. C. (1997) Crystal structure of the I domain from integrin alpha2beta1. J. Biol. Chem. **272:** 28512–28517
52 Emsley J., Knight C. G., Farndale R. W., Barnes M. J. and Liddington R. C. (2000) Structural basis of collagen recognition by integrin alpha2beta1. Cell **101:** 47–56
53 Xiong J. P., Stehle T., Zhang R., Joachimiak A., Frech M., Goodman S. L. et al. (2002) Crystal structure of the extracellular segment of integrin alpha Vbeta3 in complex with an Arg-Gly-Asp ligand. Science **296:** 151–155
54 Xiong J. P., Stehle T., Diefenbach B., Zhang R., Dunker R., Scott D. L. et al. (2001) Crystal structure of the extracellular segment of integrin alpha Vbeta3. Science **294:** 339–345
55 Vinogradova O., Velyvis A., Velyviene A., Hu B., Haas T., Plow E. et al. (2002) A structural mechanism of integrin alpha(IIb)beta(3) 'inside-out' activation as regulated by its cytoplasmic face. Cell **110:** 587
56 Weljie A. M., Hwang P. M. and Vogel H. J. (2002) Solution structures of the cytoplasmic tail complex from platelet integrin alpha IIb- and beta 3-subunits. Proc. Natl. Acad. Sci. USA **99:** 5878–5883
57 Li R., Babu C. R., Lear J. D., Wand A. J., Bennett J. S. and DeGrado W. F. (2001) Oligomerization of the integrin alphaIIbbeta3: roles of the transmembrane and cytoplasmic domains. Proc. Natl. Acad. Sci. USA **98:** 12462–12467
58 Vinogradova O., Haas T., Plow E. F. and Qin J. (2000) A structural basis for integrin activation by the cytoplasmic tail of the alpha IIb-subunit. Proc. Natl. Acad. Sci. USA **97:** 1450–1455
59 Shimaoka M., Takagi J. and Springer T. A. (2002) Conformational regulation of integrin structure and function. Annu. Rev. Biophys. Biomol. Struct. **31:** 485–516
60 Takagi J., Petre B., Walz T. and Springer T. (2002) Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. Cell **110:** 599
61 Takagi J., Erickson H. P. and Springer T. A. (2001) C-terminal opening mimics 'inside-out' activation of integrin alpha5beta1. Nat. Struct. Biol. **8:** 412–416
62 Humphries M. J. and Mould A. P. (2001) Structure. An anthropomorphic integrin. Science **294:** 316–317
63 Calderwood D. A., Yan B., de Pereda J. M., Alvarez B. G., Fujioka Y., Liddington R. C. et al. (2002) The phosphotyrosine binding-like domain of talin activates integrins. J. Biol. Chem. **277:** 21749–21758
64 Sastry S. K. and Burridge K. (2000) Focal adhesions: a nexus for intracellular signaling and cytoskeletal dynamics. Exp. Cell Res. **261:** 25–36
65 Wehrle-Haller B. and Imhof B. (2002) The inner lives of focal adhesions. Trends Cell Biol. **12:** 382
66 Miranti C. K. and Brugge J. S. (2002) Sensing the environment: a historical perspective on integrin signal transduction. Nat. Cell Biol. **4:** E83–90
67 Giancotti F. G. and Ruoslahti E. (1999) Integrin signaling. Science **285:** 1028–1032
68 Schwartz M. A. (2001) Integrin signaling revisited. Trends Cell Biol. **11:** 466–470
69 Eliceiri B. P. (2001) Integrin and growth factor receptor crosstalk. Circ. Res. **89:** 1104–1110
70 Smyth S. S. and Patterson C. (2002) Tiny dancers: the integrin-growth factor nexus in angiogenic signaling. J. Cell Biol. **158:** 17–21
71 Martin K. H., Slack J. K., Boerner S. A., Martin C. C. and Parsons J. T. (2002) Integrin connections map: to infinity and beyond. Science **296:** 1652–1653
72 Schwartz M. A. and Ginsberg M. H. (2002) Networks and crosstalk: integrin signalling spreads. Nat. Cell Biol. **4:** E65– 68
73 Schaller M. D., Hildebrand J. D., Shannon J. D., Fox J. W., Vines R. R. and Parsons J. T. (1994) Autophosphorylation of the focal adhesion kinase, pp125FAK, directs SH2- dependent binding of pp60src. Mol. Cell Biol. **14:** 1680–1688
74 Schlaepfer D. D., Hanks S. K., Hunter T. and van der Geer P. (1994) Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. Nature **372:** 786–791
75 Wary K. K., Mainiero F., Isakoff S. J., Marcantonio E. E. and Giancotti F. G. (1996) The adaptor protein Shc couples a class of integrins to the control of cell cycle progression. Cell **87:** 733–743
76 Wary K. K., Mariotti A., Zurzolo C. and Giancotti F. G. (1998) A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. Cell **94:** 625–634
77 Su B. and Karin M. (1996) Mitogen-activated protein kinase cascades and regulation of gene expression. Curr. Opin. Immunol. **8:** 402–411
78 Eliceiri B. P., Klemke R., Stromblad S. and Cheresh D. A. (1998) Integrin alphavbeta3 requirement for sustained mitogen-activated protein kinase activity during angiogenesis. J. Cell Biol. **140:** 1255–1263
79 Leppa S. and Bohmann D. (1999) Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. Oncogene **18:** 6158–6162
80 Hull C., McLean G., Wong F., Duriez P. J. and Karsan A. (2002) Lipopolysaccharide signals an endothelial apoptosis pathway through TNF receptor-associated factor 6-mediated activation of c-Jun NH2-terminal kinase. J. Immunol. **169:** 2611–2618
81 Hu Y. L., Li S., Shyy J. Y. and Chien S. (1999) Sustained JNK activation induces endothelial apoptosis: studies with colchicine and shear stress. Am. J. Physiol. **277:** H1593–599
82 Yu J., Tian S., Metheny-Barlow L., Chew L. J., Hayes A. J., Pan H. et al. (2001) Modulation of endothelial cell growth arrest and apoptosis by vascular endothelial growth inhibitor. Circ. Res. **89:** 1161–1167
83 Zhang C., Kawauchi J., Adachi M. T., Hashimoto Y., Oshiro S., Aso T. et al. (2001) Activation of JNK and transcriptional repressor ATF3/LRF1 through the IRE1/TRAF2 pathway is implicated in human vascular endothelial cell death by homocysteine. Biochem. Biophys. Res. Comm. **289:** 718–724
84 Oktay M., Wary K. K., Dans M., Birge R. B. and Giancotti F. G. (1999) Integrin-mediated activation of focal adhesion kinase is required for signaling to Jun NH2-terminal kinase and progression through the G1 phase of the cell cycle. J. Cell Biol. **145:** 1461–1469
85 Almeida E. A., Ilic D., Han Q., Hauck C. R., Jin F., Kawakatsu H. et al. (2000) Matrix survival signaling: from fibronectin via

focal adhesion kinase to c-Jun NH(2)-terminal kinase. J. Cell Biol. **149:** 741–754
86 Traub O. and Berk B. C. (1998) Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. Arterioscl. Thromb. Vasc. Biol. **18:** 677–685
87 Li S., Kim M., Hu Y. L., Jalali S., Schlaepfer D. D., Hunter T. et al. (1997) Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases. J. Biol. Chem. **272:** 30455–30462
88 Karin M., Cao Y., Greten F. R. and Li Z. W. (2002) NF-kappaB in cancer: from innocent bystander to major culprit. Nat. Rev. Cancer **2:** 301–310
89 Ghosh S. and Karin M. (2002) Missing pieces in the NF-kappaB puzzle. Cell **109 Suppl.:** S81–96
90 Shono T., Ono M., Izumi H., Jimi S. I., Matsushima K., Okamoto T. et al. (1996) Involvement of the transcription factor NF-kappaB in tubular morphogenesis of human microvascular endothelial cells by oxidative stress. Mol. Cell Biol. **16:** 4231–4239
91 Yoshida A., Yoshida S., Ishibashi T., Kuwano M. and Inomata H. (1999) Suppression of retinal neovascularization by the NF-kappaB inhibitor pyrrolidine dithiocarbamate in mice. Invest. Ophthalmol. Vis. Sci. **40:** 1624–1629
92 Scatena M., Almeida M., Chaisson M. L., Fausto N., Nicosia R. F. and Giachelli C. M. (1998) NF-kappaB mediates alphavbeta3 integrin-induced endothelial cell survival. J. Cell Biol. **141:** 1083–1093
93 Malyankar U. M., Scatena M., Suchland K. L., Yun T. J., Clark E. A. and Giachelli C. M. (2000) Osteoprotegerin is an alpha vbeta 3-induced, NF-kappa B-dependent survival factor for endothelial cells. J. Biol. Chem. **275:** 20959–20962
94 Klein S., de Fougerolles A. R., Blaikie P., Khan L., Pepe A., Green C. D. et al. (2002) Alpha 5 beta 1 integrin activates an NF-kappa B-dependent program of gene expression important for angiogenesis and inflammation. Mol. Cell Biol. **22:** 5912–5922
95 Nobes C. D. and Hall A. (1995) Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia and filopodia. Cell **81:** 53–62
96 Price L. S., Leng J., Schwartz M. A. and Bokoch G. M. (1998) Activation of Rac and Cdc42 by integrins mediates cell spreading. Mol. Biol. Cell **9:** 1863–1871
96a Ren X. D., Klosses W. B. and Schwartz M. A. (1999) Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. EMBO. J. **18:** 578–585
97 del Pozo M. A., Price L. S., Alderson N. B., Ren X. D. and Schwartz M. A. (2000) Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK. Embo J. **19:** 2008–2014
98 Seabra M. C. (1998) Membrane association and targeting of prenylated Ras-like GTPases. Cell Signal **10:** 167–172.
99 Bishop A. L. and Hall A. (2000) Rho GTPases and their effector proteins. Biochem. J. **348 Pt 2:** 241–255
100 Cotteret S. and Chernoff J. (2002) The evolutionary history of effectors downstream of Cdc42 and Rac. Genome Biol. 3.
101 Soga N., Namba N., McAllister S., Cornelius L., Teitelbaum S. L., Dowdy S. F. et al. (2001) Rho family GTPases regulate VEGF-stimulated endothelial cell motility. Exp. Cell Res. **269:** 73–87
102 Dormond O., Foletti A., Paroz C. and Ruegg C. (2001) NSAIDs inhibit alphaVbeta3 integrin-mediated and Cdc42/Rac-dependent endothelial-cell spreading, migration and angiogenesis. Nat. Med. **7:** 1041–1047
103 Kiosses W. B., Daniels R. H., Otey C., Bokoch G. M. and Schwartz M. A. (1999) A role for p21-activated kinase in endothelial cell migration. J. Cell Biol. **147:** 831–844
104 Dormond O., Bezzi M., Mariotti A. and Ruegg C. (2002) Prostaglandin E2 promotes integrin aVb3-dependent endothelial cell adhesion, Rac-activation and spreading through cAMP/PKA signaling. J. Biol. Chem. **277:** 45838–45846
105 Kiosses W. B., Shattil S. J., Pampori N. and Schwartz M. A. (2001) Rac recruits high-affinity integrin alphavbeta3 to lamellipodia in endothelial cell migration. Nat. Cell Biol. **3:** 316–320
106 Mettouchi A., Klein S., Guo W., Lopez-Lago M., Lemichez E., Westwick J. K. et al. (2001) Integrin-specific activation of Rac controls progression through the G(1) phase of the cell cycle. Mol. Cell **8:** 115–127
107 Brazil D. P. and Hemmings B. A. (2001) Ten years of protein kinase B signalling: a hard Akt to follow. Trends Biochem. Sci. **26:** 657–664
108 Lawlor M. A. and Alessi D. R. (2001) PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? J. Cell Sci. **114:** 2903–2910
109 Cantley L. C. (2002) The phosphoinositide 3-kinase pathway. Science **296:** 1655–1657
110 Gerber H. P., McMurtrey A., Kowalski J., Yan M., Keyt B. A., Dixit V. et al. (1998) Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. J. Biol. Chem. **273:** 30336–30343
111 Hermann C., Assmus B., Urbich C., Zeiher A. M. and Dimmeler S. (2000) Insulin-mediated stimulation of protein kinase Akt: a potent survival signaling cascade for endothelial cells. Arterioscler. Thromb. Vasc. Biol. **20:** 402–409
112 Kim I., Kim H. G., So J. N., Kim J. H., Kwak H. J. and Koh G. Y. (2000) Angiopoietin-1 regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway. Circ. Res. **86:** 24–29
113 Fujio Y. and Walsh K. (1999) Akt mediates cytoprotection of endothelial cells by vascular endothelial growth factor in an anchorage-dependent manner. J. Biol. Chem. **274:** 16349–16354
114 Attwell S., Roskelley C. and Dedhar S. (2000) The integrin-linked kinase (ILK) suppresses anoikis. Oncogene **19:** 3811–3815
115 Sonoda Y., Matsumoto Y., Funakoshi M., Yamamoto D., Hanks S. K. and Kasahara T. (2000) Anti-apoptotic role of focal adhesion kinase (FAK). Induction of inhibitor-of-apoptosis proteins and apoptosis suppression by the overexpression of FAK in a human leukemic cell line, HL-60. J. Biol. Chem. **275:** 16309–16315
116 Testa J. R. and Bellacosa A. (2001) AKT plays a central role in tumorigenesis. Proc. Natl. Acad. Sci. USA **98:** 10983–10985
117 Fulton D., Gratton J. P., McCabe T. J., Fontana J., Fujio Y., Walsh K. et al. (1999) Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. Nature **399:** 597–601
118 Dimmeler S., Fleming I., Fisslthaler B., Hermann C., Busse R. and Zeiher A. M. (1999) Activation of nitric oxide synthase in endothelial cells by Akt- dependent phosphorylation. Nature **399:** 601–605
119 Gratton J. P., Morales-Ruiz M., Kureishi Y., Fulton D., Walsh K. and Sessa W. C. (2001) Akt down-regulation of p38 signaling provides a novel mechanism of vascular endothelial growth factor-mediated cytoprotection in endothelial cells. J. Biol. Chem. **276:** 30359–30365
120 Meili R., Ellsworth C., Lee S., Reddy T. B., Ma H. and Firtel R. A. (1999) Chemoattractant-mediated transient activation and membrane localization of Akt/PKB is required for efficient chemotaxis to cAMP in Dictyostelium. EMBO J. **18:** 2092–2105
121 Kraynov V. S., Chamberlain C., Bokoch G. M., Schwartz M. A., Slabaugh S. and Hahn K. M. (2000) Localized Rac activation dynamics visualized in living cells. Science **290:** 333– 337
122 Lee M. J., Thangada S., Paik J. H., Sapkota G. P., Ancellin N., Chae S. S. et al. (2001) Akt-mediated phosphorylation of the

G protein-coupled receptor EDG-1 is required for endothelial cell chemotaxis. Mol. Cell **8:** 693–704
123 Genot E. M., Arrieumerlou C., Ku G., Burgering B. M., Weiss A. and Kramer I. M. (2000) The T-cell receptor regulates Akt (protein kinase B) via a pathway involving Rac1 and phosphatidylinositide 3-kinase. Mol. Cell Biol. **20:** 5469–5478
124 Higuchi M., Masuyama N., Fukui Y., Suzuki A. and Gotoh Y. (2001) Akt mediates Rac/Cdc42-regulated cell motility in growth factor- stimulated cells and in invasive PTEN knockout cells. Curr. Biol. **11:** 1958–1962
125 Chung C. Y., Potikyan G. and Firtel R. A. (2001) Control of cell polarity and chemotaxis by Akt/PKB and PI3 kinase through the regulation of PAKa. Mol. Cell **7:** 937–947
126 Byzova T. V., Goldman C. K., Pampori N., Thomas K. A., Bett A., Shattil S. J. et al. (2000) A mechanism for modulation of cellular responses to VEGF: activation of the integrins. Mol. Cell **6:** 851–860
127 Kops G. J., Medema R. H., Glassford J., Essers M. A., Dijkers P. F., Coffer P. J. et al. (2002) Control of cell cycle exit and entry by protein kinase B-regulated forkhead transcription factors. Mol. Cell Biol. **22:** 2025–2036
128 Brennan P., Babbage J. W., Burgering B. M., Groner B., Reif K. Cantrell D. A. (1997) Phosphatidylinositol 3-kinase couples the interleukin-2 receptor to the cell cycle regulator E2F. Immunity **7:** 679–689
129 Zhou B. P., Liao Y., Xia W., Spohn B., Lee M. H. and Hung M. C. (2001) Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. Nat. Cell Biol. **3:** 245–252
130 Mayo L. D. and Donner D. B. (2001) A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. Proc. Natl. Acad. Sci. USA **98:** 11598–11603
131 Rossig L., Jadidi A. S., Urbich C., Badorff C., Zeiher A. M. and Dimmeler S. (2001) Akt-dependent phosphorylation of p21(Cip1) regulates PCNA binding and proliferation of endothelial cells. Mol. Cell Biol. **21:** 5644–5657
132 Brown D. A. and London E. (2000) Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J. Biol. Chem. **275:** 17221–17224
133 Zajchowski L. D. and Robbins S. M. (2002) Lipid rafts and little caves. Compartmentalized signalling in membrane microdomains. Eur. J. Biochem. **269:** 737–752
134 Simons K. and Toomre D. (2000) Lipid rafts and signal transduction. [erratum appears in Nat. Rev. Mol. Cell Biol. 2001 Mar;2(3):216.]. Nat. Rev. Mol. Cell Biol. **1:** 31–39
135 Mariotti A., Kedeshian P. A., Dans M., Curatola A. M., Gagnoux-Palacios L. and Giancotti F. G. (2001) EGF-R signaling through Fyn kinase disrupts the function of integrin alpha6beta4 at hemidesmosomes: role in epithelial cell migration and carcinoma invasion. J. Cell Biol. **155:** 447–458
136 Leitinger B. and Hogg N. (2002) The involvement of lipid rafts in the regulation of integrin function. J. Cell Sci. **115:** 963–972
137 Thorne R. F., Marshall J. F., Shafren D. R., Gibson P. G., Hart I. R. and Burns G. F. (2000) The integrins alpha3beta1 and alpha6beta1 physically and functionally associate with CD36 in human melanoma cells. J. Biol. Chem. **275:** 35264–35275
138 Green J. M., Zhelesnyak A., Chung J., Lindberg F. P., Sarfati M., Frazier W. A. et al. (1999) Role of cholesterol in formation and function of a signaling complex involving alphavbeta3, integrin-associated protein (CD47), and heterotrimeric G proteins. J. Cell Biol. **146:** 673–682
139 Frazier W. A., Gao A. G., Dimitry J., Chung J., Brown E. J., Lindberg F. P. et al. (1999) The thrombospondin receptor integrin-associated protein (CD47) functionally couples to heterotrimeric Gi. J. Biol. Chem. **274:** 8554–8560
140 Claas C., Stipp C. S. and Hemler M. E. (2001) Evaluation of prototype transmembrane 4 superfamily protein complexes and their relation to lipid rafts. J. Biol. Chem. **276:** 7974–7984
141 Wei Y., Yang X., Liu Q., Wilkins J. A. and Chapman H. A. (1999) A role for caveolin and the urokinase receptor in integrin-mediated adhesion and signaling. J. Cell Biol. **144:** 1285–1294
142 Lacalle R. A., Mira E., Gomez-Mouton C., Jimenez-Baranda S., Martinez-A C. and Manes S. (2002) Specific SHP-2 partitioning in raft domains triggers integrin-mediated signaling via Rho activation. J. Cell Biol. **157:** 277–289
143 Karaoz E., Ilgaz C., Erdogan D. and Dagdeviren A. (1996) The expression of VLA integrins in the human thymus. Anat. Anz. **178:** 33–40
144 Korhonen M., Ylanne J., Laitinen L. and Virtanen I. (1990) The alpha 1-alpha 6 subunits of integrins are characteristically expressed in distinct segments of developing and adult human nephron. J. Cell Biol. **111:** 1245–1254
145 Zutter M. M. and Santoro S. A. (1990) Widespread histologic distribution of the alpha 2 beta 1 integrin cell- surface collagen receptor. Am. J. Pathol. **137:** 113–120
146 Sobel R. A., Hinojoza J. R., Maeda A. and Chen M. (1998) Endothelial cell integrin laminin receptor expression in multiple sclerosis lesions. Am. J. Pathol. **153:** 405–415
147 Wagner S., Tagaya M., Koziol J. A., Quaranta V. and del Zoppo G. J. (1997) Rapid disruption of an astrocyte interaction with the extracellular matrix mediated by integrin alpha 6 beta 4 during focal cerebral ischemia/reperfusion. Stroke **28:** 858–865
148 Hartner A., Schocklmann H., Prols F., Muller U. and Sterzel R. B. (1999) Alpha8 integrin in glomerular mesangial cells and in experimental glomerulonephritis. Kidney Int. **56:** 1468–1480
149 Petrova T. V., Makinen T., Makela T. P., Saarela J., Virtanen I., Ferrell R. E. et al. (2002) Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. EMBO J. **21:** 4593–4599
150 Rupp P. A. and Little C. D. (2001) Integrins in vascular development. Circ. Res. **89:** 566–572
151 Bouvard D., Brakebusch C., Gustafsson E., Aszodi A., Bengtsson T., Berna A. et al. (2001) Functional consequences of integrin gene mutations in mice. Circ. Res. **89:** 211–223
152 Yang J. T., Rayburn H. and Hynes R. O. (1993) Embryonic mesodermal defects in alpha 5 integrin-deficient mice. Development **19:** 1093–1105
153 Francis S. E., Goh K. L., Hodivala-Dilke K., Bader B. L., Stark M., Davidson D. et al. (2002) Central roles of alpha5beta1 integrin and fibronectin in vascular development in mouse embryos and embryoid bodies. Arterioscler. Thromb. Vasc. Biol. **22:** 927–933
154 Yang J. T., Rayburn H. and Hynes R. O. (1995) Cell adhesion events mediated by alpha 4 integrins are essential in placental and cardiac development. Development **121:** 549–560
155 Bader B. L., Rayburn H., Crowley D. and Hynes R. O. (1998) Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. Cell **95:** 507–519
156 McCarty J. H., Monahan-Earley R. A., Brown L. F., Keller M., Gerhardt H., Rubin K. et al. (2002) Defective associations between blood vessels and brain parenchyma lead to cerebral hemorrhage in mice lacking alphav integrins. Mol. Cell Biol. **22:** 7667–7677
157 Zhu J., Motejlek K., Wang D., Zang K., Schmidt A. and Reichardt L. F. (2002) beta8 integrins are required for vascular morphogenesis in mouse embryos. Development **129:** 2891–2903
158 Senger D. R., Claffey K. P., Benes J. E., Perruzzi C. A., Sergiou A. P. and Detmar M. (1997) Angiogenesis promoted by vascular endothelial growth factor: regulation through alpha1beta1 and alpha2beta1 integrins. Proc. Natl. Acad. Sci. USA **94:** 13612–13617

159 Senger D. R., Perruzzi C. A., Streit M., Koteliansky V. E., de Fougerolles A. R. and Detmar M. (2002) The alpha(1) beta(1) and alpha(2)beta(1) integrins provide critical support for vascular endothelial growth factor signaling, endothelial cell migration, and tumor angiogenesis. Am. J. Pathol. **160:** 195–204
160 Eliceiri B. P. and Cheresh D. A. (2000) Role of alpha v integrins during angiogenesis. Cancer J. **6 Suppl. 3:** S245–249
161 Friedlander M., Brooks P. C., Shaffer R. W., Kincaid C. M., Varner J. A. and Cheresh D. A. (1995) Definition of two angiogenic pathways by distinct alpha v integrins. Science **270:** 1500–1502
162 Paulhe F., Racaud-Sultan C., Ragab A., Albiges-Rizo C., Chap H., Iberg N. et al. (2001) Differential regulation of phosphoinositide metabolism by alphaVbeta3 and alphaVbeta5 integrins upon smooth muscle cell migration. J. Biol. Chem. **276:** 41832–41840
163 Lewis J. M., Cheresh D. A. and Schwartz M. A. (1996) Protein kinase C regulates alpha v beta 5-dependent cytoskeletal associations and focal adhesion kinase phosphorylation. J. Cell Biol. **134:** 1323–1332
164 Huang X. Z., Wu J. F., Ferrando R., Lee J. H., Wang Y. L., Farese R. V. Jr et al. (2000) Fatal bilateral chylothorax in mice lacking the integrin alpha9beta1. Mol. Cell Biol. **20:** 5208–5215
165 Frisch S. M. and Francis H. (1994) Disruption of epithelial cell-matrix interactions induces apoptosis. J. Cell Biol. **124:** 619–626
166 Meredith J. Jr, Fazeli B. and Schwartz M. A. (1993) The extracellular matrix as a cell survival factor. Mol. Biol. Cell **4:** 953–961
167 Re F., Zanetti A., Sironi M., Polentarutti N., Lanfrancone L., Dejana E. et al. (1994) Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells. J. Cell Biol. **127:** 537–546
168 Frisch S. M. and Ruoslahti E. (1997) Integrins and anoikis. Curr. Opin. Cell Biol. **9:** 701–706
169 Brooks P. C., Montgomery A. M., Rosenfeld M., Reisfeld R. A., Hu T., Klier G. et al. (1994) Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell **79:** 1157–1164
170 Frisch S. M. and Screaton R. A. (2001) Anoikis mechanisms. Curr. Opin. Cell Biol. **13:** 555–562
171 Frisch S. M., Vuori K., Kelaita D. and Sicks S. (1996) A role for Jun-N-terminal kinase in anoikis; suppression by bcl-2 and crmA. J. Cell Biol. **135:** 1377–1382
172 Stromblad S., Becker J. C., Yebra M., Brooks P. C. and Cheresh D. A. (1996) Suppression of p53 activity and p21WAF1/CIP1 expression by vascular cell integrin alphaVbeta3 during angiogenesis. J. Clin. Invest. **98:** 426–433
173 Zhang Z., Vuori K., Reed J. C. and Ruoslahti E. (1995) The alpha 5 beta 1 integrin supports survival of cells on fibronectin and up-regulates Bcl-2 expression. Proc. Natl. Acad. Sci. USA **92:** 6161–6165
174 Day M. L., Foster R. G., Day K. C., Zhao X., Humphrey P., Swanson P. et al. (1997) Cell anchorage regulates apoptosis through the retinoblastoma tumor suppressor/E2F pathway. J. Biol. Chem. **272:** 8125–8128
175 Cardone M. H., Salvesen G. S., Widmann C., Johnson G. and Frisch S. M. (1997) The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. Cell **90:** 315–323
176 Xia Z., Dickens M., Raingeaud J., Davis R. and Greenberg M. (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science **270:** 1326–1331
177 Khwaja A., Rodriguez-Viciana P., Wennstrom S., Warne P. H. and Downward J. (1997) Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. EMBO J. **16:** 2783–2793
178 Lee J. W. and Juliano R. L. (2000) alpha5beta1 integrin protects intestinal epithelial cells from apoptosis through a phosphatidylinositol 3-kinase and protein kinase B-dependent pathway. Mol. Biol. Cell **11:** 1973–1987
179 Scatena M. and Giachelli C. (2002) The alpha(v)beta3 integrin, NF-kappaB, osteoprotegerin endothelial cell survival pathway. Potential role in angiogenesis. Trends Cardiovasc. Med. **12:** 83–88
180 Tournier C., Hess P., Yang D. D., Xu J., Turner T. K., Nimnual A. et al. (2000) Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. Science **288:** 870–874
181 Frisch S. M. (1999) Evidence for a function of death-receptor-related, death-domain-containing proteins in anoikis. Curr. Biol. **9:** 1047–1049
182 Rytomaa M., Martins L. M. and Downward J. (1999) Involvement of FADD and caspase-8 signalling in detachment-induced apoptosis. Curr. Biol. **9:** 1043–1046
183 Stupack D. G., Puente X. S., Boutsaboualoy S., Storgard C. M. and Cheresh D. A. (2001) Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. J. Cell Biol. **155:** 459–470
184 Puthalakath H., Villunger A., O'Reilly L. A., Beaumont J. G., Coultas L., Cheney R. E. et al. (2001) Bmf: a proapoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis. Science **293:** 1829–1832
185 Puthalakath H., Huang D. C., O'Reilly L. A., King S. M. and Strasser A. (1999) The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. Mol. Cell **3:** 287–296
186 Aoudjit F. and Vuori K. (2001) Matrix attachment regulates Fas-induced apoptosis in endothelial cells: A role for c-Flip and implications for anoikis. J. Cell Biol. **152:** 633–643
187 Wang W. J., Kuo J. C., Yao C. C. and Chen R. H. (2002) DAP-kinase induces apoptosis by suppressing integrin activity and disrupting matrix survival signals. J. Cell Biol. **159:** 169–179
188 Kim S., Bakre M., Yin H. and Varner J. A. (2002) Inhibition of endothelial cell survival and angiogenesis by protein kinase A. J. Clin. Invest. **110:** 933–941
189 Bakre M. M., Zhu Y., Yin H., Burton D. W., Terkeltaub R., Deftos L. J. et al. (2002) Parathyroid hormone-related peptide is a naturally occurring, protein kinase A-dependent angiogenesis inhibitor. Nat. Med. **8:** 995–1003
190 Eliceiri B. P., Puente X. S., Hood J. D., Stupack D. G., Schlaepfer D. D., Huang X. Z. et al. (2002) Src-mediated coupling of focal adhesion kinase to integrin alpha(v)beta5 in vascular endothelial growth factor signaling. J. Cell Biol. **157:** 149–160
191 Bello L., Francolini M., Marthyn P., Zhang J., Carroll R. S., Nikas D. C. et al. (2001) Alpha(v)beta3 and alpha(v)beta5 integrin expression in glioma periphery. Neurosurgery **49:** 380–389
192 Brooks P. C., Clark R. A. and Cheresh D. A. (1994) Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science **264:** 569–571
193 Max R., Gerritsen R. R., Nooijen P. T., Goodman S. L., Sutter A., Keilholz U. et al. (1997) Immunohistochemical analysis of integrin alpha vbeta3 expression on tumor-associated vessels of human carcinomas. Int. J. Cancer **71:** 320–324
194 Pasqualini R., Koivunen E. and Ruoslahti E. (1997) Alpha V integrins as receptors for tumor targeting by circulating ligands. Nature Biotechnol. **15:** 542–546
195 Sipkins D. A., Cheresh D. A., Kazemi M. R., Nevin L. M., Bednarski M. D. and Li K. (1998) Detection of tumor angiogenesis in vivo by alpha V beta 3 – targeted magnetic resonance imaging. Nat. Med. **4:** 623–626
196 Stromblad S., Fotedar A., Brickner H., Theesfeld C., Aguilar de Diaz E., Friedlander M. et al. (2002) Loss of p53 compen-

sates for alpha v-integrin function in retinal neovascularization. J. Biol. Chem. **277:** 13371–13374
197 Leavesley D. I., Schwartz M. A., Rosenfeld M. and Cheresh D. A. (1993) Integrin beta 1- and beta 3-mediated endothelial cell migration is triggered through distinct signaling mechanisms. J. Cell Biol. **121:** 163–170
198 Brooks P. C., Stromblad S., Sanders L. C., von Schalscha T. L., Aimes R. T., Stetler-Stevenson W. G. et al. (1996) Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. Cell **85:** 683–693
199 Soldi R., Mitola S., Strasly M., Defilippi P., Tarone G. and Bussolino F. (1999) Role of alphavbeta3 integrin in the activation of vascular endothelial growth factor receptor-2. EMBO J. **18:** 882–892
200 Cheresh D. A. (1987) Human endothelial cells synthesize and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor. Proc. Natl. Acad. Sci. USA **84:** 6471–6475
201 Dechantsreiter M. A., Planker E., Matha B., Lohof E., Holzemann G., Jonczyk A. et al. (1999) N-Methylated cyclic RGD peptides as highly active and selective alpha(V)beta(3) integrin antagonists. J. Med. Chem. **42:** 3033–3040
202 Hammes H. P., Brownlee M., Jonczyk A., Sutter A. and Preissner K. T. (1996) Subcutaneous injection of a cyclic peptide antagonist of vitronectin receptor-type integrins inhibits retinal neovascularization. Nature Med. **2:** 529–533
203 Brooks P. C., Stromblad S., Klemke R., Visscher D., Sarkar F. H. and Cheresh D. A. (1995) Antiintegrin alpha v beta 3 blocks human breast cancer growth and angiogenesis in human skin. J. Clin. Invest. **96:** 1815–1822
204 MacDonald T. J., Taga T., Shimada H., Tabrizi P., Zlokovic B. V., Cheresh D. A. et al. (2001) Preferential susceptibility of brain tumors to the antiangiogenic effects of an alpha(v) integrin antagonist. Neurosurgery **48:** 151–157
205 Boger D. L., Goldberg J., Silletti S., Kessler T. and Cheresh D. A. (2001) Identification of a novel class of small-molecule antiangiogenic agents through the screening of combinatorial libraries which function by inhibiting the binding and localization of proteinase MMP2 to integrin alpha(V)beta(3). J. Am. Chem. Soc. **123:** 1280–1288
206 Posey J. A., Khazaeli M. B., DelGrosso A., Saleh M. N., Lin C. Y., Huse W. et al. (2001) A pilot trial of Vitaxin, a humanized anti-vitronectin receptor (anti alpha v beta 3) antibody in patients with metastatic cancer. Cancer Biother. Radiopharm. **16:** 125–132
207 Reynolds L. E., Wyder L., Lively J. C., Taverna D., Robinson S. D., Huang X. et al. (2002) Enhanced pathological angiogenesis in mice lacking beta3 integrin or beta3 and beta5 integrins. Nat. Med. **8:** 27–34
208 Tomiyama Y. (2000) Glanzmann thrombasthenia: integrin alpha IIb beta 3 deficiency. Int. J. Hematol. **72:** 448–454
209 Hynes R. O. and Hodivala-Dilke K. M. (1999) Insights and questions arising from studies of a mouse model of Glanzmann thrombasthenia. Thrombosis Haemostasis **82:** 481–485
210 McHugh K. P., Hodivala-Dilke K., Zheng M. H., Namba N., Lam J., Novack D. et al. (2000) Mice lacking beta3 integrins are osteosclerotic because of dysfunctional osteoclasts. J. Clin. Invest. **105:** 433–440
211 Horton M. A. (2001) Integrin antagonists as inhibitors of bone resorption: implications for treatment. Proc. Nutr. Soc. **60:** 275–281
212 Kim S., Bell K., Mousa S. A. and Varner J. A. (2000) Regulation of angiogenesis in vivo by ligation of integrin alpha5beta1 with the central cell-binding domain of fibronectin. Am. J. Pathol. **156:** 1345–1362
213 Diaz-Gonzalez F., Forsyth J., Steiner B. and Ginsberg M. H. (1996) Trans-dominant inhibition of integrin function. Mol. Biol. Cell **7:** 1939–1951
214 Cheresh D. A. and Stupack D. G. (2002) Integrin-mediated death: an explanation of the integrin-knockout phenotype? Nat. Med. **8:** 193–194
215 Hynes R. O. (2002) A reevaluation of integrins as regulators of angiogenesis. Nat. Med. **8:** 918–921
216 Lawler J. (2000) The functions of thrombospondin-1 and-2. Curr. Opin. Cell Biol. **12:** 634–640
217 Iruela-Arispe M. L., Vazquez F. and Ortega M. A. (1999) Antiangiogenic domains shared by thrombospondins and metallospondins, a new family of angiogenic inhibitors. Ann. N. Y. Acad. Sci. **886:** 58–66
218 Bello L., Lucini V., Carrabba G., Giussani C., Machluf M., Pluderi M. et al. (2001) Simultaneous inhibition of glioma angiogenesis, cell proliferation, and invasion by a naturally occurring fragment of human metalloproteinase-2. Cancer Res. **61:** 8730–8736
219 Pfeifer A., Kessler T., Silletti S., Cheresh D. A. and Verma I. M. (2000) Suppression of angiogenesis by lentiviral delivery of PEX, a noncatalytic fragment of matrix metalloproteinase 2. Proc. Natl. Acad. Sci. USA **97:** 12227–12232
220 Brooks P. C., Silletti S., von Schalscha T. L., Friedlander M. and Cheresh D. A. (1998) Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity. Cell **92:** 391–400
221 Maeshima Y., Yerramalla U. L., Dhanabal M., Holthaus K. A., Barbashov S., Kharbanda S. et al. (2001) Extracellular matrix-derived peptide binds to alpha(v)beta(3) integrin and inhibits angiogenesis. J. Biol. Chem. **276:** 31959–31968
222 Maeshima Y., Sudhakar A., Lively J. C., Ueki K., Kharbanda S., Kahn C. R. et al. (2002) Tumstatin, an endothelial cell-specific inhibitor of protein synthesis. Science **295:** 140–143
223 Shiff S. J. and Rigas B. (1999) The role of cyclooxygenase inhibition in the antineoplastic effects of nonsteroidal antiinflammatory drugs (NSAIDs). J. Exp. Med. **190:** 445–450
224 Gupta R. A. and DuBois R. N. (2001) Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat. Cancer Rev. **1:** 11–21
225 Williams C. S., Tsujii M., Reese J., Dey S. K. and DuBois R. N. (2000) Host cyclooxygenase-2 modulates carcinoma growth. J. Clin. Invest. **105:** 1589–1594
226 Tsujii M., Kawano S., Tsuji S., Sawaoka H., Hori M. and DuBois R. N. (1998) Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell **93:** 705–716
227 Murohara T., Horowitz J. R., Silver M., Tsurumi Y., Chen D., Sullivan A. et al. (1998) Vascular endothelial growth factor/vascular permeability factor enhances vascular permeability via nitric oxide and prostacyclin. Circulation **97:** 99–107
228 Jones M. K., Wang H., Peskar B. M., Levin E., Itani R. M., Sarfeh I. J. et al. (1999) Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. Nat. Med. **5:** 1418–1423
229 Muller-Decker K., Neufang G., Berger I., Neumann M., Marks F. and Furstenberger G. (2002) Transgenic cyclooxygenase-2 overexpression sensitizes mouse skin for carcinogenesis. Proc. Natl. Acad. Sci. USA **99:** 12483–12488
230 Liu C. H., Chang S. H., Narko K., Trifan O. C., Wu M. T., Smith E. et al. (2001) Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J. Biol. Chem. **276:** 18563–18569
231 Xu J., Rodriguez D., Petitclerc E., Kim J. J., Hangai M., Yuen S. M. et al. (2001) Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth in vivo. J. Cell Biol. **154:** 1069–1079
232 Davis G. E. (1992) Affinity of integrins for damaged extracellular matrix: alpha v beta 3 binds to denatured collagen type I through RGD sites. Biochem. Biophys. Res. Commun. **182:** 1025–1031

233 Shang M., Koshikawa N., Schenk S. and Quaranta V. (2001) The LG3 module of laminin-5 harbors a binding site for integrin alpha3beta1 that promotes cell adhesion, spreading, and migration. J. Biol. Chem. **276:** 33045–33053

234 Giannelli G., Falk-Marzillier J., Schiraldi O., Stetler-Stevenson W. G. and Quaranta V. (1997) Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. Science **277:** 225–228

235 O'Reilly M. S., Boehm T., Shing Y., Fukai N., Vasios G., Lane W. S. et al. (1997) Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell **88:** 277–285

236 Rehn M., Veikkola T., Kukk-Valdre E., Nakamura H., Ilmonen M., Lombardo C. et al. (2001) Interaction of endostatin with integrins implicated in angiogenesis. Proc. Natl. Acad. Sci. USA **98:** 1024–1029

237 Dvorak H. F., Nagy J. A., Feng D., Brown L. F. and Dvorak A. M. (1999) Vascular permeability factor/vascular endothelial growth factor and the significance of microvascular hyperpermeability in angiogenesis. Curr. Topics. Microbiol. Immunol. **237:** 97–132

238 Eliceiri B. P., Paul R., Schwartzberg P. L., Hood J. D., Leng J. and Cheresh D. A. (1999) Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. Mol. Cell. **4:** 915–924

239 Castellani P., Dorcaratto A., Pau A., Nicola M., Siri A., Gasparetto B. et al. (2000) The angiogenesis marker ED-B+ fibronectin isoform in intracranial meningiomas. Acta. Neurochir. **142:** 277–282

240 Tokes A. M., Hortovanyi E., Kulka J., Jackel M., Kerenyi T. and Kadar A. (1999) Tenascin expression and angiogenesis in breast cancers. Pathol. Res. Practice **195:** 821–828

241 Murphy-Ullrich J. E. (2001) The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state? J. Clin. Invest. **107:** 785–790

242 Pasqualini R., Koivunen E. and Ruoslahti E. (1995) A peptide isolated from phage display libraries is a structural and functional mimic of an RGD-binding site on integrins. J. Cell Biol. **130:** 1189–1196

243 Kon S., Yokosaki Y., Maeda M., Segawa T., Horikoshi Y., Tsukagoshi H. et al. (2002) Mapping of functional epitopes of osteopontin by monoclonal antibodies raised against defined internal sequences. J. Cell Biochem. **84:** 420–432

244 Yokosaki Y., Matsuura N., Higashiyama S., Murakami I., Obara M., Yamakido M. et al. (1998) Identification of the ligand binding site for the integrin alpha9 beta1 in the third fibronectin type III repeat of tenascin-C. J. Biol. Chem. **273:** 11423–11428

245 Ruoslahti E. and Rajotte D. (2000) An address system in the vasculature of normal tissues and tumors. Ann. Rev. Immunol. **18:** 813–827

246 Arap W., Pasqualini R. and Ruoslahti E. (1998) Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science **279:** 377–380

247 Sipkins D. A., Cheresh D. A., Kazemi M. R., Nevin L. M., Bednarski M. D. and Li K. C. (1998) Detection of tumor angiogenesis in vivo by alphaVbeta3-targeted magnetic resonance imaging. Nat. Med. **4:** 623–626

248 Hood J. D., Bednarski M., Frausto R., Guccione S., Reisfeld R. A., Xiang R. et al. (2002) Tumor regression by targeted gene delivery to the neovasculature. Science **296:** 2404–2407

249 Wickham T. J., Mathias P., Cheresh D. A. and Nemerow G. R. (1993) Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. Cell **73:** 309–319

250 Guerrero C. A., Mendez E., Zarate S., Isa P., Lopez S. and Arias C. F. (2000) Integrin alpha(v)beta(3) mediates rotavirus cell entry. Proc. Natl. Acad. Sci. USA **97:** 14644–14649

251 Berinstein A., Roivainen M., Hovi T., Mason P. W. and Baxt B. (1995) Antibodies to the vitronectin receptor (integrin alpha V beta 3) inhibit binding and infection of foot-and-mouth disease virus to cultured cells. J. Virol. **69:** 2664–2666

252 Vigne E., Mahfouz I., Dedieu J. F., Brie A., Perricaudet M. and Yeh P. (1999) RGD inclusion in the hexon monomer provides adenovirus type 5-based vectors with a fiber knob-independent pathway for infection. J. Virol. **73:** 5156–5161

253 Lienard D., Ewalenko P., Delmotte J. J., Renard N. and Lejeune F. J. (1992) High-dose recombinant tumor necrosis factor alpha in combination with interferon gamma and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. J. Clin. Oncol. **10:** 52–60

254 Lejeune F., Rüegg C. and Liénard D. (1998) Clinical applications of TNF-alpha in cancer. Curr. Opin. Immunol. **10:** 573–580

255 Ruegg C., Dormond O., Oguey D. and Lejeune F. J. (2000) Tumor necrosis factor: clinical use and mechanisms of action. Drug Res. Updates **3:** 271–276

256 Yilmaz A., Bieler G., Spertini O., Lejeune F. J. and Ruegg C. (1998) Pulse treatment of human vascular endothelial cells with high doses of tumor necrosis factor and interferon-gamma results in simultaneous synergistic and reversible effects on proliferation and morphology. Int. J. Cancer **77:** 592–599

257 Dormond O., Lejeune F. and Rüegg C. (2002) TNF and IFNgamma induced G1/S block in human endothelial cells is associated with expression of p16INK4a. Anticancer Res. **22:** in press

258 Ruegg C., Yilmaz A., Bieler G., Bamat J., Chaubert P. and Lejeune F. J. (1998) Evidence for the involvement of endothelial cell integrin alpha-v-beta-3 in the disruption of the tumor vasculature induced by tnf and ifn-gamma. Nat. Med. **4:** 408–414

259 LaFlamme S. E., Thomas L. A., Yamada S. S. and Yamada K. M. (1994) Single subunit chimeric integrins as mimics and inhibitors of endogenous integrin functions in receptor localization, cell spreading and migration, and matrix assembly. J. Cell Biol. **126:** 1287–1298

260 Lukashev M. E., Sheppard D. and Pytela R. (1994) Disruption of integrin function and induction of tyrosine phosphorylation by the autonomously expressed beta 1 integrin cytoplasmic domain. J. Biol. Chem. **269:** 18311–18314

261 Oguey D., George P. W. and Rüegg C. (2000) Disruption of integrin-dependent adhesion and survival of endothelial cells by recombinant adenovirus expressing isolated beta integrin cytoplasmic domains. Gene Ther. **7:** 1292–1303

262 Liu K. Y., Timmons S., Lin Y. Z. and Hawiger J. (1996) Identification of a functionally important sequence in the cytoplasmic tail of integrin beta 3 by using cell-permeable peptide analogs. Proc. Natl. Acad. Sci. USA **93:** 11819–11824

263 Eigenthaler M., Hofferer L., Shattil S. J. and Ginsberg M. H. (1997) A conserved sequence motif in the integrin beta3 cytoplasmic domain is required for its specific interaction with beta3-endonexin. J. Biol. Chem. **272:** 7693–7698

264 Klinghoffer R. A., Sachsenmaier C., Cooper J. A. and Soriano P. (1999) Src family kinases are required for integrin but not PDGFR signal transduction. EMBO J. **18:** 2459–2471

265 Ilic D., Furuta Y., Kanazawa S., Takeda N., Sobue K., Nakatsuji N. et al. (1995) Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature **377:** 539–544

266 Sieg D. J., Hauck C. R. and Schlaepfer D. D. (1999) Required role of focal adhesion kinase (FAK) for integrin-stimulated cell migration. J. Cell Sci. **112:** 2677–2691

267 Datta S. R., Brunet A. and Greenberg M. E. (1999) Cellular survival: a play in three Akts. Genes Dev. **13:** 2905–2927

268 Yoganathan T. N., Costello P., Chen X., Jabali M., Yan J., Leung D. et al. (2000) Integrin-linked kinase (ILK): a 'hot' therapeutic target. Biochem. Pharmacol. **60:** 1115–1119

269 Guba M., von Breitenbuch P., Steinbauer M., Koehl G., Flegel S., Hornung M. et al. (2002) Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat. Med. **8:** 128–135
270 Dormond O. and Rüegg C. (2001) Inhibition of tumor angiogenesis by non-steroidal anti inflammatory drugs: emerging mechanisms and therapeutic perspectives. Drug Res. Updates **4:** 314–321
271 Evers E. E., Zondag G. C., Malliri A., Price L. S., ten Klooster J. P., van der Kammen R. A. et al. (2000) Rho family proteins in cell adhesion and cell migration. Eur. J. Cancer **36:** 1269–1274
272 Welsh C. F. and Assoian R. K. (2000) A growing role for Rho family GTPases as intermediaries in growth factor- and adhesion-dependent cell cycle progression. Biochim. Biophys. Acta **1471:** M21–29