Integrins, angiogenesis and vascular cell survival

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The interactions between integrins and the extracellular matrix have been identified as important regulators of vascular cell survival, proliferation and invasion during the complex process of blood vessel formation by angiogenesis.

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Angiogenesis is the mechanism by which new blood vessels are formed from pre-existing vessels; this is distinct from vasculogenesis, where vessels develop from mesodermal angioblast precursor cells [1]. The mechanism of angiogenesis can generally be divided into three phases, as shown for tumor-induced angiogenesis in Figure 1.

The formation of new blood vessels facilitates physiological processes of embryonic development, female reproduction and wound healing [2]. However, angiogenesis also has a critical role in various pathological conditions such as solid tumor formation, metastasis, childhood hemangiomas, macular degeneration, diabetic retinopathy, psoriasis and inflammation-related diseases such as rheumatoid arthritis, ulcerative colitis and osteoarthritis [2]. In particular, the expansion of solid tumors beyond a minimal size is critically dependent on the formation of new blood vessels to supply oxygen, nutrients and growth factors, but angiogenesis is also crucial for the formation of metastases at secondary sites [2]. Accordingly, inhibitors of angiogenesis have been suggested as therapeutic tools in the treatment of cancer [2]. The members of one group of angiogenesis inhibitors that will soon be tested in clinical trials are antagonists of a_v integrins. This group includes genetically engineered monoclonal antibodies, synthetic linear and cyclic peptides, as well as naturally occurring venoms.

Integrins in angiogenesis

Integrins are a family of heterodimeric transmembrane adhesion receptors that mediate cell–extracellular matrix interactions, such as cell adhesion and migration, and in some cases cell–cell adhesion [3]. The integrins and their respective ligands are summarized in Table 1.

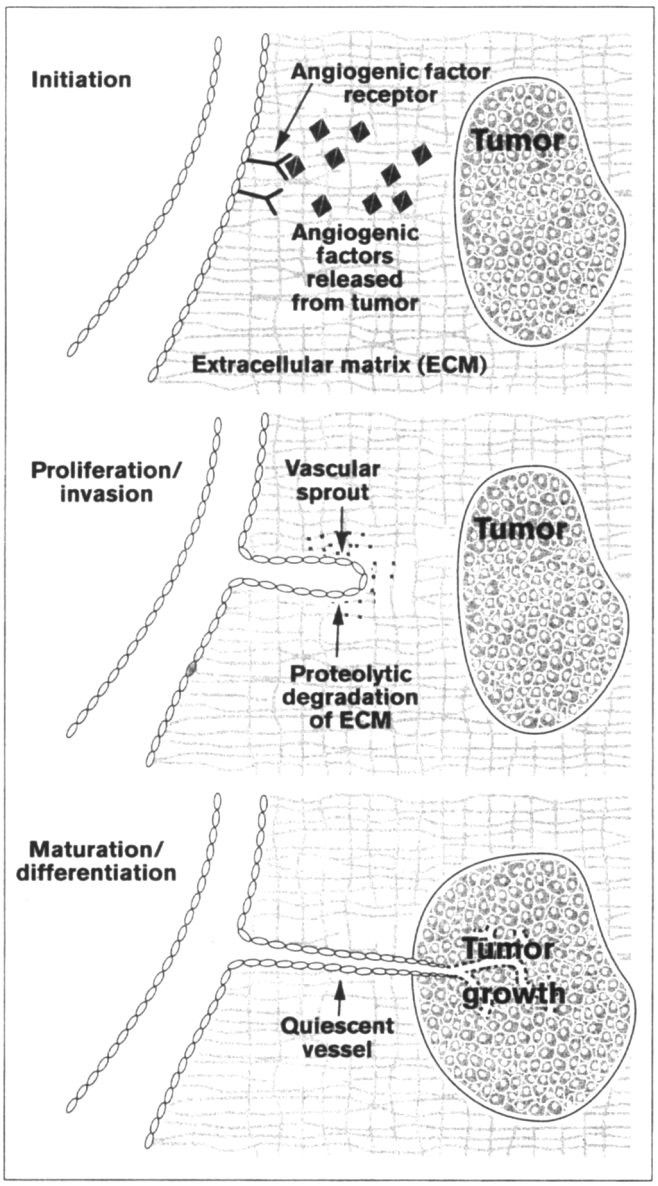
Endothelial cells express several distinct integrins, allowing attachment to a wide spectrum of extracellular-matrix (ECM) components including fibronectin, vitronectin, laminin, collagen types I and IV, von Willebrand factor, fibrinogen and denatured collagen. The functions of integrins in endothelial cell biology have been studied in various *in vitro* and *in vivo* models. *In vitro*, one of the laminin receptors, integrin $\alpha_6\beta_1$, has been implicated in capillary tube formation [4], a model involving endothelial cell migration and differentiation [5,6]. Function-blocking antibodies against either α_6 or β_1 blocked tube formation, whereas antibodies against α_5 did not [4]. This is consistent with a previously proposed role for laminin in endothelial cell differentiation into capillary tubes [5,6].

Integrin-mediated signaling events in endothelial cells have also been studied using immobilized anti-integrin antibodies as agonists. While both β_1 and $\alpha_v\beta_3$ integrins mediate increases in intracellular pH, only $\alpha_v\beta_3$ is capable of mediating calcium influx [7]. However, conflicting results have been presented regarding endothelial cell tube formation using functional blocking antibodies against integrin $\alpha_2\beta_1$ and $\alpha_v\beta_3$, since both have been reported to enhance as well as block tube formation [8–10]. These confusing findings underscore the problems of exclusively examining models of angiogenesis *in vitro*, as the results are not necessarily predictive of the effects on angiogenesis *in vivo*.

In vivo, knockout of the gene encoding the fibronectin receptor α_5 subunit resulted in multiple lethal defects in early embryonic development, including blood vessel formation, similar to what is observed in fibronectin knockouts (reviewed in [11]). However, $\alpha_v\beta_3$ is the integrin that has been studied most extensively during angiogenesis *in vivo*. Integrin $\alpha_v\beta_3$ is preferentially expressed on angiogenic blood vessels, and subsequent blockage of $\alpha_v\beta_3$ function by antibody or peptide antagonists prevents blood vessel formation in the chick chorioallantoic membrane (CAM), quail embryo, rabbit cornea, mouse retina and in human skin transplanted onto SCID mice (mice with severe combined immunodeficiency) [12–17]. In fact, this blockage of angiogenesis also results in an inhibition of tumor growth of $\alpha_v\beta_3$-negative human tumors in the chick embryo (by inhibiting chick angiogenesis), and in human skin transplants in SCID mice (by inhibiting human angiogenesis within the skin) [13,14]. Notably, function blocking of $\alpha_v\beta_3$ during angiogenesis leads to apoptosis selectively in proliferating angiogenic vessels [13]. This suggests that integrin $\alpha_v\beta_3$ has a unique function during angiogenesis, namely to provide specific survival signals that will facilitate vascular cell proliferation.

Integrin $\alpha_v\beta_3$ can interact with a wide variety of ECM proteins (Table 1). We recently showed that integrin $\alpha_v\beta_3$ can also bind directly to matrix metalloproteinase-2

Figure 1



Tumor-induced angiogenesis. Angiogenesis, the formation of new blood vessels from pre-existing vessels, can generally be divided into three phases, as exemplified by tumor-induced angiogenesis. First, angiogenic stimulators such as bFGF and VEGF are released from the tumor and/or inflammatory cells. These angiogenic signals trigger the proliferating and invading phase, characterized by vascular cell proliferation, secretion of proteolytic enzymes and extracellular matrix (ECM) molecules as well as altered expression of adhesion molecules. The proteolytic enzymes act to degrade extracellular matrix proteins, which together with new synthesis of ECM molecules results in a remodeling of the extracellular microenvironment. This remodeling, together with the altered adhesive properties of the vascular cells, is critical as it facilitates vascular cell survival, proliferation and migration, resulting in a vascular invasion of the ECM and the tumor. Finally, the vascular sprouts begin to mature by forming differentiated luminal structures, and to fuse into loops, thereby facilitating blood circulation in the new vessels.

(MMP-2), thereby localizing MMP-2 mediated matrix degradation capacity to the vascular cell surface during angiogenesis [18]. These observations may shed light on how integrins and proteases function in a coordinated manner to promote cell invasion during angiogenesis and other cellular invasion events.

While antagonists of $\alpha_v\beta_3$ can block tumor-induced angiogenesis, individuals suffering from a form of Glanzmann thrombobasthenia have apparently normal blood vessels, despite lacking the integrin β_3 subunit [19]. It can be noted, however, that these patients are severely compromised in their wound-repair function. People with this disorder may develop blood vessels in the absence of $\alpha_v\beta_3$ by an alternative mechanism using integrin $\alpha_v\beta_5$, which can potentiate a distinct pathway of angiogenesis [16].

Recent pharmacological and toxicological studies indicate that antagonists of $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ are well tolerated in different species, suggesting that these antagonists might be safe for therapeutical purposes. In fact, clinical trials will soon be started for evaluation of humanized monoclonal antibodies against integrin $\alpha_v\beta_3$ as an anti-angiogenic therapy for cancer. Other antagonists of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ that are of potential clinical use are various Arg-Gly-Asp (RGD)-containing snake venoms, linear RGD peptides, as well as other short peptide sequences discovered using phage display libraries [20,21]. In addition, antagonists with high affinity and specificity for α_v integrins have been developed in the form of cyclic peptides, such as RGD-fV, where f is the D-form of phenylalanine [22].

Cell adhesion and apoptosis

Integrins not only participate in cell adhesion and cytoskeletal re-organization events necessary for cell migration, but also mediate transmembrane signaling from the ECM into the cell [23,24]. Integrin-induced signals include H^+ exchange, Ca^{2+} influx, tyrosine, serine and threonine phosphorylation events, as well as altered phosphoinositide metabolism. In many cases, these signals have been linked to the regulation of gene expression and contribute to mechanisms such as proliferation, differentiation and cell survival. Recent studies have demonstrated that integrin-mediated cell adhesion can also prevent apoptosis, a form of programmed cell death [13,25–27].

Apoptosis is an evolutionary conserved process by which cells actively commit suicide, and it is critical for tissue homeostasis and the regulation of development and disease [28]. Adhesion-dependent proliferation is tightly regulated in most non-transformed cells, so a role for integrin–ECM interactions in regulating apoptosis is well suited. However, studies in distinct cell systems under different conditions also suggest a role for diverse integrins as mediators of cell survival. Thus, the particular integrin mediating cell survival might be cell type- and

Table 1

Integrins and their ligands and/or counterreceptors

Subunits		Ligands and/or counter-receptors
β_1	α_1	Collagens, laminin
	α_2	Collagens, laminin
	α_3	Fibronectin, laminin, collagens, epiligrin, entactin
	α_4	Fibronectin, VCAM-1
	α_5	Fibronectin, L1-CAM, tenascin, invasin
	α_6	Laminin, merosin, kalinin, invasin
	α_7	Laminin
	α_8	Tenascin, fibronectin, vitronectin
	α_9	Collagen type I, laminin, tenascin
	α_v	Vitronectin, fibronectin, osteopontin
β_2	α_L	ICAM-1, ICAM-2, ICAM-3
	α_M	ICAM-1, fibrinogen, factor X, C3b
	α_X	Fibrinogen, C3b
β_3	α_{IIb}	Fibrinogen, fibronectin, vitronectin, von Willebrand factor
	α_v	Vitronectin, osteopontin, fibrinogen, von Willebrand factor, fibronectin, denatured collagen types I, IV and VI, bone sialoprotein, thrombospondin, PECAM-1, L1-CAM, fibrillin-1, tenascin
β_4	α_6	Laminin, kalinin
β_5	α_v	Vitronectin, osteopontin, fibronectin
β_6	α_v	Fibronectin
β_7	α_4	Fibronectin, VCAM-1, MadCAM-1
	α_E	?
β_8	α_v	Fibronectin, vitronectin

Integrins are a family of transmembrane heterodimeric adhesion receptors composed of one α and one β subunit. As indicated above, some of the integrin subunits are capable of forming dimers with more than one corresponding subunit. The integrins mediate cell–extracellular matrix interactions and in some cases cell–cell interactions, and are capable of transmitting various signals into the cell [20,21]. The table is an updated adaption from [3].

condition-specific. This may in part be explained by the distinct roles of different extracellular matrix components. For example, provisional matrices, such as fibronectin, vitronectin and fibrinogen, typically potentiate cell proliferation, whereas the basement membrane promotes cell cycle withdrawal and differentiation [29,30]. Therefore, attachment to different ECM components is mediated through specific integrins, that can generate distinct signals that contribute to cell survival in multiple ways (Table 1). To this end, Boudreau and colleagues [31] recently demonstrated a relationship between the type of ECM interaction and the specific survival signals in mammary cells. Differentiation of mammary epithelial cells was induced by interaction with a three-dimensional basement membrane type matrix and accompanied by down-regulation of c-myc and cyclin D1, which normally promote proliferation. Once these cells became differentiated and quiescent, re-expression of c-myc resulted in apoptosis. By contrast, high expression levels of c-myc and cyclin D1 in proliferating mammary cells were not accompanied by apoptosis [31]. Furthermore, overexpression of $p21^{WAF-1/CIP-1}$, a cell-cycle-arrest molecule that is highly expressed during differentiation, led to apoptosis when overexpressed in the proliferating mammary cells [31]. These findings couple the regulation of cell survival by the ECM intimately to cell-cycle control, and suggest that introduction of signals that are in conflict with those mediated through the integrins may result in apoptosis. Not surprisingly, cell-cycle progression is also controlled by cell adhesion, linked to adhesion-dependent regulation of cyclin A and the cyclin-dependent kinase (cdk)-inhibitors $p21^{WAF-1/CIP-1}$ and $p27^{KIP-1}$ [32,33].

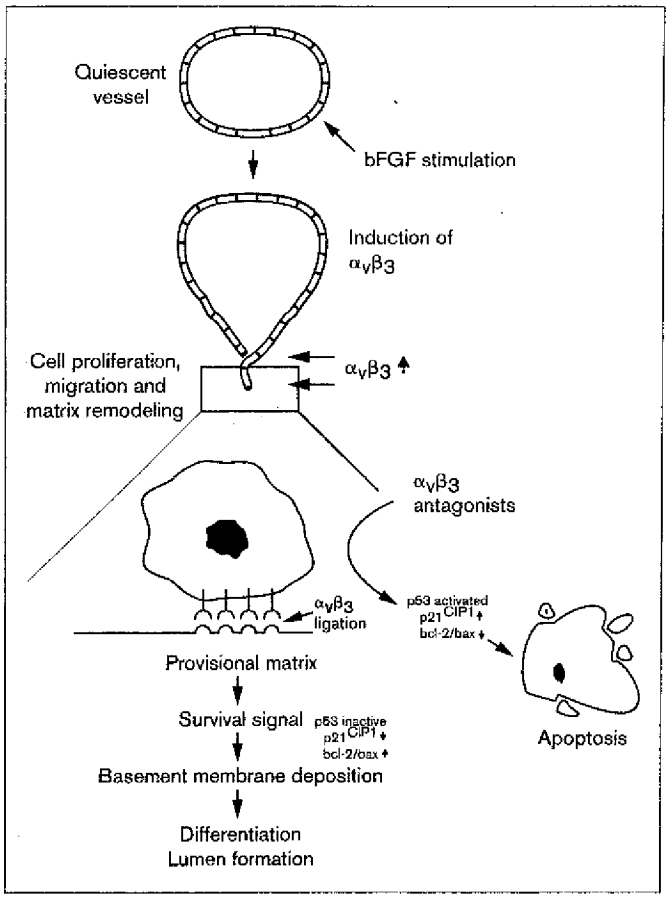
Integrins and vascular cell survival

Primary endothelial cells are anchorage-dependent and undergo apoptosis when denied integrin-mediated attachment, indicating that integrins regulate endothelial cell survival *in vitro* [25,34]. However, these studies do not address integrin specificity or whether these events take place in a complex three dimensional extracellular matrix environment *in vivo*. Blocking integrin $\alpha_v\beta_3$ results in the induction of apoptosis among proliferating vascular cells as described above [13]. Specific antibody or cyclic peptide antagonists of $\alpha_v\beta_3$ administered systemically during angiogenesis on the chick CAM also induce endothelial cell p53-dependent DNA binding activity, whereas antibodies to integrin β_1 have no effect on p53 activity or apoptosis [13,35]. This induction of p53 activity is accompanied by an increased expression of the p53-inducible cell-cycle inhibitor $p21^{WAF-1/CIP-1}$. Furthermore, using immobilized anti-integrin antibodies as agonists of integin function *in vitro*, it has been shown that ligation of cultured human endothelial cell $\alpha_v\beta_3$, but not other integrins, is sufficient to suppress p53 activity without changing p53 protein expression. The reduced p53 activity is accompanied by a sharp decrease in $p21^{WAF-1/CIP-1}$ protein levels, demonstrating that the ligation state of integrin $\alpha_v\beta_3$ directly regulates both of the cell cycle inhibitors p53 and $p21^{WAF-1/CIP-1}$ [35]. Ligation of $\alpha_v\beta_3$ also enhanced Bcl-2 expression while decreasing that of Bax. The resulting increase in the Bcl-2:Bax ratio has been shown previously to promote cell survival [28]. Thus, ligand binding by endothelial cell integrin $\alpha_v\beta_3$ suppresses apoptosis and conflicting growth-arrest signals, thereby facilitating the proliferation and maturation of new blood vessels during angiogenesis.

It is not clear whether activation of the cell-cycle inhibitors p53 and $p21^{WAF-1/CIP-1}$ by $\alpha_v\beta_3$ antagonists is the direct cause of apoptopsis during angiogenesis. However, integrin $\alpha_v\beta_3$ mediates adhesion to proteins that typically comprise a provisional matrix, such as proteolysed collagen, vitronectin, fibronectin and fibrinogen. As these matrix components promote cell proliferation [29,30], it might be expected that ligand binding to $\alpha_v\beta_3$ would in turn suppress the activity of molecules such as p53 and $p21^{WAF-1/CIP-1}$, which otherwise could inhibit

cell-cycle progression and induce apoptosis [31,35,36]. This suggests a model in which integrin–extracellular matrix interactions are necessary to coordinate the activity of both cell-cycle promotors and inhibitors during angiogenesis (Fig. 2). Thus, during endothelial cell proliferation, an interaction between $\alpha_v\beta_3$ and the provisional ECM would suppress conflicting growth-arrest signals, such as p53 and $p21^{WAF-1/CIP-1}$ [35,36]. However, to secure vascular cell survival during differentiation, the basement membrane would coordinately downregulate molecules that promote cell-cycle progression when cell-cycle inhibitors become activated. This may avoid a potential conflict that otherwise would lead to apoptosis, as has been observed when c-myc is introduced into mammary epithelial cells that are allowed to differentiate on a basement membrane [31].

Figure 2



Hypothetical model for the role of integrin $\alpha_v\beta_3$ in angiogenesis. Angio-genic stimulus by bFGF induces expression of integrin $\alpha_v\beta_3$ and causes cells to invade the surrounding ECM and to enter the cell cycle. If ligation of $\alpha_v\beta_3$ is blocked, endothelial cell p53 activity is induced, accompanied by increased $p21^{WAF-1/CIP-1}$ expression, a decrease in the bcl-2/bax ratio and apoptosis of proliferating vascular cells [13,32]. This suggests a model where the interaction between integrins and the extracellular matrix regulates proliferation and survival. To facilitate cell prolifer-ation and survival, the interaction between $\alpha_v\beta_3$ and the provisional ECM suppresses potentially conflicting growth arrest signals by blocking p53 activity and $p21^{WAF-1/CIP-1}$ expression [32]. This enables vessel maturation associated with the formation of an intact basement membrane, leading to final endothelial differentiation and lumen formation.

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