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Cell adhesion molecules in angiogenesis

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Angiogenesis is the complex process by which new blood vessels are generated from preexisting vessels. This process is different from the related cellular event of vasculogenesis, in that blood vessels develop from precursor cells called angioblasts [1, 2]. The growth of new blood vessels plays a critical roles in a variety of normal physiological events including embryonic development, endometrial proliferation and wound healing [3–5]. However. many pathological processes are also characterized by abnormal vascular development such as arthritis, diabetic retinopathy and tumor growth and metastasis [3–5].

In particular, the expansion of solid tumors is critically dependent on vascular networks to provide nutrients for growing tumors. In fact, in order for tumors to grow beyond a minimal size they require an extensive blood supply. Furthermore, the degree of vascularization of many solid tumors correlates with a poor clinical prognosis and an increased likelihood of metastatic disease [6–8]. Therefore, the process of tumor angiogenesis may be a clinically relevant target for therapeutic intervention.

To begin to identify molecules that play a functional role in angiogenesis, we must first understand the cellular processes that lead to tumor neovascularization. These cellular events can be categorized into three generalized stages (Figure 1). Initiation events involve release of angiogenic molecules and cytokines from both tumor cells and associated inflammatory cells. These growth factors bind to their cognate receptors leading to transmembrane signaling events that activate the resting blood vessels. The proliferative and invasive phases of angiogenesis are characterized by an increase in cellular mitogenesis, expression of cell adhesion molecules and secretion of proteolytic enzymes. The increased proteolytic activity is of critical importance since degradation of the extracellular matrix provides a permissive microenvironment in which activated vascular cells proliferate, invade and migrate away from the preexisting parental vessel. It is well established that the processes of cell adhesion, migration, invasion and proliferation are mediated in part by cell adhesion molecules. These facts implicate cell adhesion receptors as critical players in the angiogenic cascade. The maturation phase of angiogenesis also involves cell-cohesive events such as cell-cell contacts and cell-extracellular matrix (ECM) interactions that eventually lead to capillary lumen formation. These receptor ligand interactions may lead to a cascade of biochemical signals ultimately resulting in differentiation of pre-capillary sprouts into mature tumor associated vessels.

In recent years numerous studies have concentrated on identifying molecules involved in the initiation phase of tumor angiogenesis [9, 10]. These studies have led to the conclusions that tumor cells and inflammatory cells such as macrophages and mast cells can release angiogenic molecules, including basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and tumor necrosis factor alpha (TNF-α). Furthermore, it has been suggested that extracellular matrix bound cytokines released by proteolysis may contribute to angiogenic stimulation [11, 12].

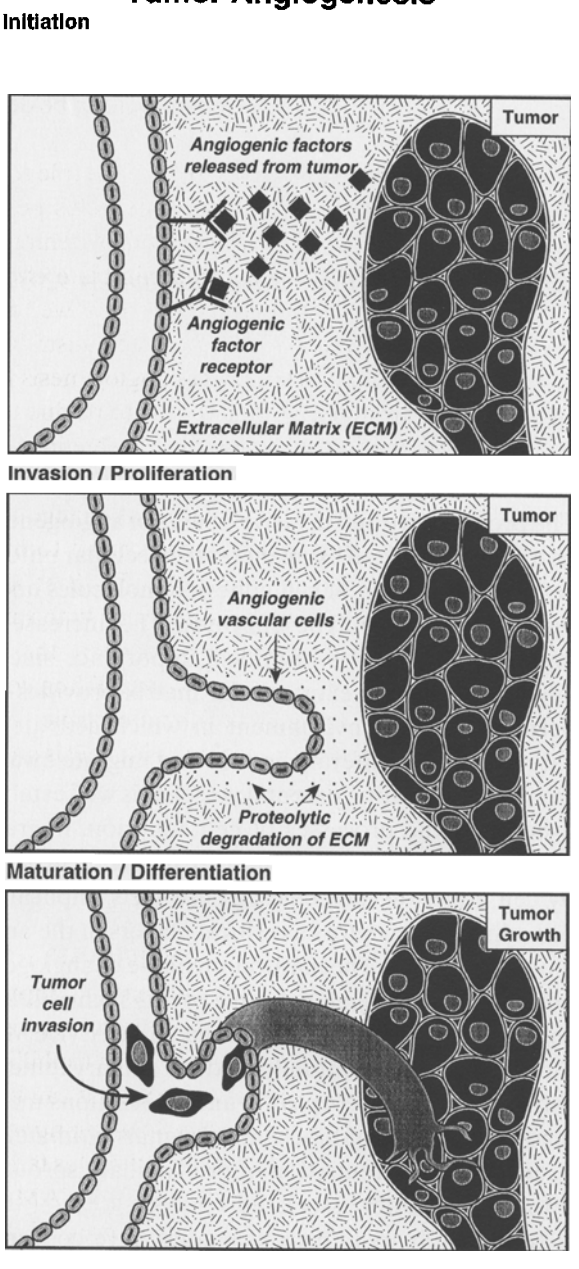

Figure 1. Tumor angiogenesis. The biochemical and cellular events that occur during angiogenesis can be grouped into three stages: Initiation, Invasion/Proliferation and Maturation/Differentiation. Initiation: Growth factors and cytokines released from both tumor cells and associated inflammatory cells interact with endothelial cell surface receptors. This receptor-ligand interaction causes activation of the normally quiescent vascular endothelium. Invasion/Proliferation: Activated vascular endothelial cells begin to proliferate and express increased levels of specific cell adhesion molecules and matrix altering proteinases. The degradation and modification of the extracellular matrix (ECM) creates a permissive microenvironment for vascular cell invasion (migration) and proliferation. Maturation/Differentiation: cell-cell and cell-ECM interactions promote distinct biochemical signals necessary for lumen formation and differentiation of capillary sprouts into mature tumor associated blood vessels.

Until recently, little was known concerning the signal transduction pathways involved in angiogenesis. However, a number of endothelial specific tyrosine kinase receptors have been implicated in angiogenesis and vascular development, including flt-1, and Flk/KDR, two known receptors for VEGF [13, 14]. Moreover, two additional novel tyrosine kinase receptors Tie-1 and Tie-2 have been identified that also appear to be expressed in endothelial cells [15]. Recent studies involving gene knock out mice have implicated these four receptors in biochemically distinct signaling pathways associated with angiogenesis and vascular development [13–15]. These studies and others are beginning to address the complex signaling events associated with angiogenesis.

This review will focus primarily on a discussion of the potential roles of cell adhesion molecules in angiogenesis and tumor neovascularization (Table 1). Cell adhesion molecules can be classified into at least four distinct families depending on their biochemical and structural characteristics. These families include the selectins, the immunoglogulin supergene family, the cadherins and the integrin family of cell adhesion receptors. These families possess specific structural and sequence characteristics that may promote distinct cell adhesive and biochemical signaling events.

The selectin family

Selectins are a group of cell adhesion molecules of which at least three distinct members are known including L-selectin, P-selectin and E-selectin. These transmembrane molecules have been shown to mediate cell-cell interactions. This family is characterized by conserved cystein residues, a lectin like domain which mediate interactions with complex carbohydrate moieties, and tandem repeat sequences

similar to those found in complement-binding proteins [16–18]. P-selectin and E-selectin recognize sialylated glycans such as sialyl-Lewis X (SLex) and sialyl-Lewis A (SLea) containing molecules. Members of this family have been shown to be differentially expressed on cytokine activated vascular endothelium. Specifically, both P-selectin and E-selectin can be upregulated on endothelial cells after treatment with inflammatory mediators or cytokines such as LPS, TNFα and IL1β [18].

Furthermore, both P-selectin and E-selectin can be expressed in at least two distinct forms including a transmembrane and soluble form [18].

Recent studies by Nguyen et al. have implicated E-selectin and sialyl-Lewis X/A glyconjugates in capillary tube formation [19]. In this study, specific antibodies directed to either sialyl-Lewis X or sialyl-Lewis A antigens inhibited bovine capillary tube formation *in vitro*. In contrast, antibodies directed to Lewis X, A or H had no effect. These results suggest a role for sialylated glycan containing ligands in capillary morphogenesis. E-selectin and P-selectin have been shown to bind to sialyl Lewis X/A glycoconjugates, therefore studies were performed to determine the roles of these selectins in capillary tube formation. Nguyen et al. showed that specific antibodies directed to E-selectin could block capillary tube formation whereas, antibodies directed to P-selectin had no effect [19]. Thus, it appears that E-selectin sialyl Lewis-X/A interaction may potentiate capillary morphogenesis *in vitro*. These findings suggest a functional role for E-selectin in tumor angiogenesis since capillary tube formation is a critical event during angiogenesis. However, the down stream signaling and cell adhesive events mediated by this receptor ligand interaction remains to be determined.

Further evidence supporting a functional role for selectins in angiogenesis comes from studies performed by Koch et al. [20]. These studies demonstrate that soluble E-selectin could stimulate chemotaxis of human endothelial cells *in vitro* as well as inducing angiogenesis *in vivo*. The mechanism by which soluble E-selectin stimulates angiogenesis is not clear. However, it does not appear to be due to mitogenic stimulation since soluble E-selectin did not induce proliferation of the endothelial cells tested [20]. Furthermore, no significant change in production of a variety of cytokines known to induce angiogenesis was detected [20]. Koch et al. suggest that soluble E-selectin interaction with sialyl-Lewis X containing ligand may promote these angiogenic and chemotactic responses. Taken together these studies suggest a functional role for members of the selectin family in angiogenesis and vascular development.

Table 1. Cell adhesion molecules in angiogenesis

	References
Selectin family	
E-selectin	Ref: 19, 20
Immunoglobulin family	
VCAM-1	Ref: 20
Integrin family	
β1	Ref: 39, 41, 42, 51
α5	Ref: 40
α6	Ref: 39
αvβ3	Ref: 38, 48, 49, 50, 51, 53, 54
αvβ5	Ref: 54

Cell adhesion molecules suggested to play a role in angiogenesis.

The immunoglobulin supergene family

The immunoglobulin supergene family (IgSF) is a second group of adhesion molecules recently implicated in angiogenesis. These transmembrane molecules have been shown to mediate heterophillic cell-cell adhesion. All members of this class can be characterized by repetitive extracellular immunoglobulin like domains [17, 21]. Members of this family include ICAM-1, ICAM-2, ICAM-3, VCAM-1 and PECAM. Whereas ICAM-2 is expressed at high levels on resting endothelial cells, ICAM-1 and VCAM-1 are variably expressed on quiescent endothelium, but are upregulated after stimulation with cytokines such as TNF-α, IL-1 or INF-γ [17]. Both ICAM-1 and VCAM-1 are expressed in two forms including a transmembrane and soluble form. ICAM-1 has been shown to bind to the leukocyte integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), whereas VCAM-1 serves as a counter receptor for VLA-4 integrin (CD49d/CD29) [17]. In recent studies by Koch et al. it was shown that soluble

VCAM-1 can induce chemotaxis in human endothelial cells *in vitro* and induce angiogenesis *in vivo* [20] in a manner comparable to E-selectin. However, soluble VCAM-1 did not induce mitogenesis of endothelial cells or upregulate cytokines known to induce angiogenesis [20]. To further study the possible mechanism involved in VCAM-1 induced chemotaxis and angiogenesis, antibodies directed to VLA-4 integrin, a known receptor for soluble VCAM-1, were tested for its effects on VCAM-1 stimulated endothelial cell chemotaxis. Antibodies directed to VLA-4 significantly reduce VCAM-1 stimulated motility [20]. These results suggest a possible role of VLA-4 integrin in soluble VCAM-1 mediated biological responses. It is possible that interactions between soluble VCAM-1 and VLA-4 may transmit specific signals necessary for endothelial cell motility. In fact, it has been demonstrated that ligation of β1 integrins can regulate cell adhesion and motility of endothelial cells [22]. Furthermore, it has been shown that the cytoplasmic tails of β1 integrins can interact with specific molecules thought to be involved in signal transduction such as focal adhesion kinase [23]. Thus, at least one member of the immunoglobulin supergene family may play a role in angiogenesis. Further study of this class of adhesion molecules may reveal other members that also contribute to angiogenesis and vascular development.

The cadherin family

The cadherin family of adhesion molecules include members such as E-cadherin, P-cadherin and L-cadherin [24, 25]. These molecules have a set of common features that distinguish them from other cell adhesion receptors. These molecules share a highly conserved cytoplasmic domain that may interact with several cytosolic proteins such as α, β and γ catenins [21, 26]. In addition, these molecules have structurally similar extracellular domains that can bind cadherins on adjacent cells in a calcium dependent manner. These molecules mediate calcium dependent homophillic cell-cell interactions, possibly leading to assembly of intracellular tight junctions, gap junctions and desosomes [27]. These biologically distinct structures may be important in maintaining the integrity of both developing and mature blood vessels. In fact, Liaw et al. have identified two distinct cadherin species expressed in bovine endothelial cells with homologies to N-cadherin and P-cadherin [28]. Furthermore, Salmon et al. have shown that human endothelial cells express N-cadherin diffusely on the cell surface with occasional cell-cell junctional staining [29]. While no direct evidence implicates these molecules in angiogenesis, it is intriguing to speculate a possible role for cadherins in the later differentiation stages of angiogenesis involved in cell-cell contacts and lumen formation. Alternatively, the loss of cadherins in early angiogenesis may promote increased invasion and motility of activated endothelial cells as has been demonstrated in the case of invasive tumor cells [30]. However, the potential role of any of the cadherin family of cell adhesion molecules awaits further investigation.

The integrin family

The fourth major category of cell adhesion molecules is the integrin family. Integrins are a group of cell adhesion receptors that are composed of non-covalently associated α and β chains that combine to give a wide variety of heterodimers with distinct cellular and adhesive specificities [31]. These transmembrane cell adhesion receptors predominately mediate cell-ECM interactions, however some members can potentate cell-cell adhesive events. To date their are at least 15 α and 8 β subunits which can combine to give at least 20 distinct integrins [32]. The unique combination of alpha and beta chains dictates in part their ligand specificity. These multifunctional adhesion receptors have been shown to regulate a wide variety of cellular and biochemical responses including, adhesion, migration, invasion, proliferation, apoptosis and gene expression [33–36].

Members of the integrin family are expressed on a wide variety of cells and tissues including endothelial cells. In fact, members of the β1 sub-family including α1β1, α2β1, α3β1, α4β1, α5β1 and α6β1 are all expressed on endothelial cells. However, some

receptors, such as αvβ3 are only minimally expressed on quiescent blood vessels, but are highly upregulated during angiogenesis [38].

Several members of this family have recently been implicated in angiogenesis and tumor neovascularization including members of the β1, β3 and β5 sub-families. Studies by Bauer et al. show that antibodies directed to either α6 or β1 integrins could block capillary tube formation *in vitro*, whereas antibodies to α5 integrin had only minimal effects [39]. However, caution must be used in extrapolating results from *in vitro* tube forming assays to *in vivo* angiogenesis. For example, whereas α5 integrin appeared to play little if any functional role in capillary tube formation *in vitro*, studies by Yang et al. using α5 gene knock out mice show numerous defects in blood vessel formation [40]. These contrasting results may be explained by the complexity of cellular processes functioning *in vivo* as compared to *in vitro* tube forming assays. Further support for a role of β1 integrins in vascular development comes from studies by Grant et al. which implicate β1 integrin interaction with laminin in regulating capillary tube lumen formation [41]. Finally, studies by Drake et al. focus on the role of β1 integrins in vasculogenesis in the quail embryo. Injection of antibodies directed to β1 integrins significantly disrupted vascular development and lumen formation [42]. Although these studies were directed at vasculogenesis rather than angiogenesis, it does however provides further support for a role of β1 integrins in cell adhesive processes involved in vascular development. It is interesting to speculate on the possible mechanism by which β1 integrins may potentiate angiogenesis. For example, it is known that β1 integrins can mediate cell-cell and cell ECM interactions. Therefore, it is possible that these cellular cohesive events are required for the structural integrity necessary for lumen formation. Alternatively, blocking β1 integrin-ligand interactions could effect endothelial cell migration on ECM components such as laminin, fibronectin and collagen. In fact, it has been shown by Ingber et al. and Haralabopoulos et al. that cellular interactions with collagen may be important since disrupting normal collagen synthesis and deposition can significantly inhibit angiogenesis [43, 44]. These results have physiological significance since its been demonstrated that fibronectin, collagen and laminin are present in the extracellular matrix surrounding angiogenic blood vessels. A third possibility involves the induction of matrix metalloproteinase expression by β1 integrin ligation [45]. It is known that members of both the matrix metalloproteinases as well as serine proteinases may be required for neovascularization as inhibitors of these enzymes can block angiogenesis both *in vitro* and *in vivo* [46, 47]. Further research into the roles of β1 integrins in angiogenesis will help clarify these complex possibilities.

Recent studies have identified integrin αvβ3 as a marker of angiogenic and proliferating blood vessels [38]. In fact, only minimal if any αvβ3 is expressed in normal resting blood vessels. However, cytokine activated and tumor associated vessels express high levels of this integrin. Moreover, recent studies demonstrate a functional role for integrin αvβ3 in both cytokine and tumor induced angiogenesis. Antibodies directed to integrin αvβ3 inhibited angiogenesis induced by cytokines or tumor fragments *in vivo* [48]. In addition, a cyclic RGD peptide known to interact with αvβ3 and αvβ5 also blocked human tumor induced angiogenesis in the chick embryo whereas control RAD peptides had no effect. In studies performed by Nicosia et al., synthetic RGD peptides which could also disrupt integrin αvβ3-ligand interactions inhibited microvessel growth from rings of rat aorta embedded in collagen gels [49]. Finally, in separate studies, systemic administration of monoclonal antibody LM609 directed to integrin αvβ3 inhibited human breast tumor induced angiogenesis and tumor proliferation in human skin transplanted on SCID mice [50].

Further evidence supporting a role for integrin αvβ3 in angiogenesis comes from studies by Davis et al. Polyclonal antibodies directed to integrin αvβ3 or monoclonal antibodies specific to αv, β3 and β1 integrins blocked endothelial cord formation in matrigel cultures *in vitro* [51]. In contrast, Gamble and colleagues showed that antibodies to integrin αvβ3 actually enhanced tube formation in fibrin gel cultures [52]. These conflicting results again may be explained by the different *in vitro* sys-

tems utilized. It may be that in the purified fibrin gel system, cell adhesive and biochemical signaling events are different as compared to the more complex nature of the matrigel system. These differences could lead to distinct biochemical and cellular responses. In fact, it is known that the microenvironment of a given cell can significantly effect its cellular behavior.

Recent studies by Drake et al. provide further evidence that integrin αvβ3 plays a significant role in neovascularization and blood vessel maturation [53]. Monoclonal antibody LM609 directed to integrin αvβ3 was microinjected into developing quail embryos. Treatment of these embryos resulted in a significant disruption pre-capillary lumen formation and abnormal vascular patterning, demonstrating the importance of αvβ3 for this process.

Finally, studies by Friedlander et al. further implicated integrin αvβ3 in angiogenesis in the rabbit corneal model [55]. Antibodies directed to integrin αvβ3 were copolymerized with bFGF in hydron pellets implanted into rabbit corneas. Antibodies to integrin αvβ3 significantly inhibited angiogenesis induced by bFGF whereas antagonists of integrin αvβ5 had no effect [54]. Taken together these studies strongly suggest a functional role for integrin αvβ3 in angiogenesis and tumor neovascularization. Recent studies by Brooks et al. have suggested a potential mechanism to account for the inhibition of angiogenesis by antagonists of integrin αvβ3 *in vivo* [48]. These studies demonstrated that antagonists of integrin αvβ3 could specifically induce apoptosis or programmed cell death in angiogenic blood vessels. Furthermore, in studies by Stromblad et al. it was shown that ligation of vascular integrin αvβ3 in angiogenic endothelial cells suppressed p53 activity and increased bcl-2/bax ratio, both known regulators of apoptosis [S. Stromblad & D. Cheresh, personal cummunication]. These results suggest that integrin αvβ3 may be functioning in latter events during the angiogenic cascade.

A second vitronectin receptor, integrin αvβ5 has recently been implicated in angiogenesis. In studies by Friedlander et al. in the rabbit corneal and chick embryo models, antibodies directed to integrin αvβ5 significantly block angiogenesis induced VEGF, TGF-α, and PMA, whereas antagonists of integrin αvβ3 had only minimal effects on angiogenesis induced by these cytokines [54]. In contrast, antagonists of integrin αvβ3 blocked angiogenesis induced by both bFGF and TNF-α. These results imply that specific cytokines may stimulate angiogenesis by distinct signaling pathways that may in part be defined by specific αv integrins. Further studies are required to define the potential role of these distinct cytokine mediated angiogenic pathways in tumor neovascularization. These results further demonstrate the complexity of angiogenesis and the numerous adhesive proteins that may regulate this process *in vivo*.

Other cell adhesive proteins

Cell adhesion molecules that do not fit exclusively into one of the discrete categories mentioned previously, such as the nonintegrin 32kd/67kd laminin binding protein may also contribute to angiogenesis [41]. In studies by Grant et al. antibodies directed to nonintegrin laminin binding protein significantly blocked capillary tube formation in matrigel culturs of endothelial cells. These studies suggest that 32/67 kd laminin binding protein may be involved in differentiation of endothelial cells into capillary like tubes [41].

Conclusions

Elegant studies on the biological role of cell adhesion molecules are leading to the emergence of these molecules as critical players in many normal physiological and pathological processes. A growing interest in the biochemical and cellular events regulating tumor neovascularization has lead to studies implicating these molecules in angiogenesis. The cell adhesion molecules discussed in this review are not meant to represent an all inclusive list of adhesion receptors involved in angiogenesis, as new member of this expanding group will likely be added. However, these molecules represent current examples of cell adhesion receptors that have been clearly implicated in neovascular development. As work in the fields of cell adhesion and biochemical

signaling merge, progress will likely reveal novel molecules that contribute to the regulation angiogenesis and tumor neovascularization. Further studies are likely to bring new insight into the possible biochemical mechanisms regulating this complex process. Furthermore, an active on going discussion of the role of cell adhesion molecules in tumor neovascularization may stimulate interest in these molecules as potential therapeutic targets for the treatment for neoplastic diseases.

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