Cell adhesion and angiogenesis.

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Perspective

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Cell Adhesion in Vascular Biology

Cell Adhesion and Angiogenesis

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Introduction

Angiogenesis is the growth of new capillary blood vessels from preexisting capillaries and postcapillary venules. This process is critical for normal growth and development and in protective responses such as wound healing and inflammation. In healthy adults, angiogenesis does not normally occur except in certain phases of the female reproductive cycle. However, aberrant angiogenesis can occur in a variety of pathologic settings. These include the neovascularization of solid tumors, the growth of vessels into the retina in diabetic retinopathy, and the unwanted vessel growth in chronic inflammatory diseases. The hypothesis that angiogenic diseases, in particular tumor growth and metastases, may be alleviated by inhibiting the angiogenic responses (1) has prompted many to investigate the basic mechanisms by which endothelial cells are stimulated to undergo angiogenesis. The goal is to identify biochemical events that constitute potential targets for antiangiogenic therapy (2). A recent strategy has been to identify endogenous angiogenesis inhibitors and use these molecules to control angiogenesis.

Endothelial cell proliferation is a major component of angiogenesis, but is only one of a series of tasks the endothelial cells must accomplish to form a new capillary blood vessel. In response to angiogenic stimuli, endothelial cells degrade the extracellular matrix (ECM),[1] migrate into the perivascular space, proliferate, and align themselves into patent blood vessels. When sufficient angiogenesis has occurred, the endothelial cells become quiescent and the vessels either remain or regress if no longer needed. During these events, the endothelial cells must adhere to one another and to the ECM to construct and extend new microvessels. The endothelial cells also form branches which then connect to other branches to form capillary loops. The polarity of the endothelial cell, luminal versus abluminal, must also be established. Pericytes, which are thought to regulate function and growth, are found associated with the abluminal surfaces of mature capillary blood vessels

and postcapillary venules (3). Thus, the formation of a new microvessel requires a number of interactions that must be coordinated in a spatially and temporally specified manner. These adhesion events are likely mediated by endothelial cell adhesion molecules and ECM molecules that provide instructions to the endothelial cells as they migrate into the perivascular space and assemble into new vessels with surrounding pericytes.

Cell adhesion and endothelial cell growth

Adhesion of endothelial cells to ECM and attainment of an appropriate cellular shape has been known for many years to be crucial for endothelial cell growth, differentiation, and survival. In 1978, Folkman and Moscona used poly(2-hydroxyethyl methacrylate) to vary the adhesiveness of plastic culture dishes in order to measure the effect of cell adhesion and shape on proliferation of bovine aortic endothelial cells (4). Increased cell spreading, determined by measuring cell diameter and height, was found to be tightly coupled to increased [^{3}H]thymidine incorporation and cellular proliferation. Thus, the shape of the cells, and not just attachment of cells to the substratum, is a critical parameter for growth. Similar results were obtained when varying densities of fibronectin were used to modulate shape of bovine capillary endothelial cells in serum-free conditions (5). In subsequent studies, nonadherent endothelial cells in suspension were found to undergo programmed cell death (6). The programmed cell death (i.e., apoptosis) could be prevented by plating the endothelial cells on fibronectin-coated or anti–β_1 integrin-coated dishes but not dishes coated with anti–vascular cell adhesion molecule-1 antibodies. These studies indicate that integrin binding to ECM ligands plays a crucial role in endothelial cell growth and survival. The consequences of disrupting requisite adhesion events may be twofold. Endothelial cells in a sprouting vessel may become misguided and unable to assemble into a new capillary blood vessel and/or the cells may undergo programmed cell death in response to the lack of appropriate cell contacts. The mechanism(s) by which the extracellular environment affects endothelial cell adhesion, shape, and proliferation is a central question in angiogenesis research.

Endothelial cell adhesion molecules

To date, four families of cell adhesion molecules have been described: integrins, immunoglobulin superfamily members, cadherins, and selectins. Members of each family have been detected in angiogenic blood vessels. The integrin family of heterodimeric adhesion molecules functions in a variety of cell–matrix and cell–cell interactions in the vasculature. Several integrins are expressed on luminal and abluminal surfaces of endothelial cells and have been shown to participate in en-

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1. *Abbreviations used in this paper:* CAM, chorioallantoic membrane; ECM, extracellular matrix.

dothelial cell migration and formation of capillary-like tubes in vitro (7). In vivo, integrins have been shown to be involved in both angiogenesis and vasculogenesis. One particular complex, the αvβ3 integrin, has been studied extensively in a number of in vivo models in which angiogenesis can be induced. αvβ3 is detected in growing but not quiescent blood vessels and is also expressed on a number of other cell types including tumor cells, smooth muscle cells, fibroblasts, and leukocytes. Furthermore, αvβ3 can bind an array of ligands such as vitronectin, fibronectin, von Willebrand factor, fibrinogen, osteopontin, thrombospondin, and RGD-containing peptides. Recently, αvβ3 integrin expressed on lymphokine-activated killer cells has been shown to bind PECAM-1/CD31 (8) and αvβ3 expressed on melanoma cells has been shown to bind matrix metalloproteinase-2 (9), despite the lack of RGD sequences in these two polypeptides. Thus, αvβ3 can be expressed in many cell types and may bind to a diverse array of ligands in many settings, including but not limited to angiogenesis.

The importance of αvβ integrins in angiogenesis was first elucidated by Cheresh and colleagues in a series of experiments (10, 11) using an anti-αvβ3 mAb designated LM609 and a cyclic RGD-peptide antagonist of αv-integrins. The LM609 mAb has proved to be a valuable and essential reagent for their investigations in that LM609 reacts with several species including human, chick, quail, and rabbit αvβ3 integrins, it is highly specific for the heterodimeric complex, and it can react with the complex in a variety of experimental conditions.

In the chick chorioallantoic membrane (CAM) assay, an in vivo assay for angiogenesis, LM609 mAb inhibited blood vessel growth induced by implanting an αvβ3-negative melanoma or induced by implanting a pellet containing basic fibroblast growth factor (10). The effect was specific in that although β1 integrins are expressed in CAM blood vessels, anti-β1 mAb had no effect on the angiogenic responses. The importance of αv integrins in angiogenesis has been confirmed using a cyclic RGD peptide antagonist for αvβ3 and αvβ5. The cyclic RGD peptide inhibits tumor-induced angiogenesis in the CAM (11) and hypoxia-induced neovascularization in the murine retina (12). In follow-up studies, antibody against αvβ5 was found to inhibit angiogenesis induced with vascular endothelial cell growth factor, transforming growth factor-α, or phorbol 12-myristate 13-acetate (13). In contrast, αvβ3 was required when angiogenesis was stimulated with basic fibroblast growth factor or TNF-α. These results demonstrate that the integrins used to produce new blood vessels can differ depending on the angiogenic stimuli. This suggests that antiangiogenic agents may be selective for certain angiogenic responses, depending on the mechanism(s) used in a given angiogenic disease or process.

Besides its role in experimentally induced angiogenesis, αvβ3 integrin has been shown to function in embryonic neovascularization. When three or four somite stage quail embryos were injected with the anti-αvβ3 mAb LM609, vessel lumens failed to form and a notable disruption in overall blood vessel pattern was observed (14). In this study, Drake and colleagues (14) showed that αvβ3 could be immunoprecipitated from quail embryos, that LM609 bound to endothelial cells in whole-mount embryos, and that vitronectin was colocalized with αvβ3 at the basal surface of nascent vessels. This suggests that vitronectin may bind to αvβ3 during embryonic neovascularization, an important observation in view of the fact that ligand(s) with which αv-integrins interact during experimentally induced angiogenesis have not been defined.

The mechanism by which anti-αvβ3 mAb disrupts angiogenesis appears to involve apoptosis since LM609-positive blood vessels were found colocalized with apoptotic cells (11). This finding suggests that proper ligation of αvβ3 to its endogenous ligand is important for maintaining the appropriate angiogenic signal to endothelial cells. In the absence of such signals, proliferating endothelial cells respond by initiating programmed cell death. In support of this, disruption of αvβ3 binding in proliferating endothelial cells was found to result in expression of conflicting cellular signals in that p53 activity and cell cycle inhibitor p21[waf1/cip1] were induced (15). (p53 expression in proliferating cells has been shown to trigger apoptosis.) Interestingly, attachment of human umbilical vein endothelial cells to the LM609 mAb immobilized on tissue culture dishes rescued cells from expression of p53 activity and p21[waf1/cip1]. Thus, the same antibody that exerts dramatic antiangiogenic effects in vivo can prevent expression of growth inhibitory activities in vitro. This suggests that cell attachment via αvβ3 is required for maintaining an angiogenic program in endothelial cells. It would be of interest to analyze endothelial cells plated on poly(2-hydroxyethyl methacrylate) or fibronectin, as described in early studies (4, 5), to determine the pattern of p53 and p21[waf1/cip1] expression in spread versus round endothelial cells.

An important question is whether loss of cell adhesion, either to matrix or adjacent cells, is a critical step by which angiogenesis is switched off and vessel regression ensues, in either normal development or in pathological angiogenesis. Signals that trigger apoptosis, whether due to loss of cell contact, as shown with αvβ3, or loss of an angiogenic factor, are likely to be important since apoptotic endothelial cells have been detected during blood vessel involution in rat retinal endothelial cells after exposure to hyperoxia (16) and involutive phase infantile hemangiomas (Razon, M.J., B.M. Kräling, J.B. Mulliken, and J. Bischoff, manuscript in preparation).

Cadherins, selectins, and immunoglobulin superfamily members
Besides integrins, a number of other cell adhesion molecules have been suggested to function in angiogenesis. In some cases, the suggestion comes from observations that the expression of a particular adhesion molecule coincides with angiogenesis and/or vascular development. For example, VE-cadherin mRNA and polypeptide are detected in endothelial precursors in murine embryos beginning at E7.5 (17). Furthermore, VE-cadherin polypeptide is expressed throughout vascular development and is localized at points of cell–cell contact. These observations make it attractive to speculate that it plays a role in angiogenesis, perhaps by specifying endothelial cell organization and/or differentiation into mature vessels (18). PECAM-1/CD31 has also been proposed to play a role in angiogenesis based on its expression in angiogenic blood vessels and its localization (19).

Some lines of experimental evidence suggest that E-selectin, a member of the selectin family of adhesion molecules, participates in angiogenesis. E-Selectin in an endothelial membrane glycoprotein best known for its ability to promote adhesion of leukocytes to cytokine-activated endothelial cells. The hypothesis that E-selectin may also function in angiogenesis is based on in vitro observations that antibodies directed against either E-selectin or sialylated fucosylated oligosaccharides, structures to which E-selectin can bind, inhibit the formation of capillary-like tubes in vitro (20). These endothelial cells ex-

press increased levels of sialylated fucosylated oligosaccharides (21). Furthermore, carbohydrate analogues of sialylated Lewis-X prevent human umbilical vein endothelial cells from forming capillary-like tubes (22). We have also demonstrated that E-selectin is expressed in proliferating endothelial cells in infantile hemangioma tumors and in other noninflammatory angiogenic tissues such as human placenta (23). Furthermore, the levels of E-selectin in hemangiomas, but not P-selectin, correlate with angiogenesis. Thus, E-selectin is present at the right place and time to participate in angiogenesis.

Exogenously added soluble E-selectin has also been shown to induce angiogenesis in the rat cornea (24), a widely used in vivo model for angiogenesis, and to stimulate chemotaxis of human endothelial cells in vitro. The chemotaxis was blocked by anti-sialylated Lewis-X mAb indicating that E-selectin interacts with a sialylated Lewis X–containing ligand on endothelial cells. A soluble recombinant form of vascular cell adhesion molecule-1 was also shown to have angiogenic activity in this study but required much higher concentrations compared with the recombinant E-selectin. This series of experiments presents an intriguing possibility that shed adhesion molecules stimulate angiogenesis in vivo. In summary, E-selectin is associated with essential components of angiogenesis, endothelial cell proliferation, migration, capillary tube formation, and neovascularization. However, the mechanism by which E-selectin contributes to angiogenesis is likely to be subtle since E-selectin–deficient mice are viable and reproduce normally (25, 26). Given the essential role the microvasculature plays in organ development and function, compensatory molecules may be upregulated in E-selectin–deficient mice. Mice deficient in both E- and P-selectins are also viable, indicating that P-selectin does not compensate for E-selectin in angiogenesis (26). Another point to consider is that E-selectin may not play a central role in mice. In vitro studies of microvascular endothelial cells from wild-type mice indicate that only low levels of murine E-selectin polypeptide are expressed (27). Although its function in angiogenic blood vessels is unclear, several properties of E-selectin may be exploited for angiogenesis research. First, E-selectin is one of the few adhesion molecules that is truly restricted to endothelium. Therefore, antibodies directed against E-selectin can be used to detect, isolate, and quantitate proliferating and/or cytokine-activated endothelial cells. Second, the expression of E-selectin in cytokine-stimulated and/or proliferating endothelial cells may be used to selectively target activated and/or proliferating endothelium in vivo.

In summary, with the exception of the αv integrins, relatively little is known about the mechanism by which cell adhesion molecules function in angiogenesis. More studies will be required to decipher the potential roles of E-selectin, VE-cadherin, and to identify new adhesion molecules that function in angiogenesis. It is clear that endothelial cells require adhesive interactions to attain an appropriate cellular configuration for growth, survival, and differentiation. Given this and the numerous studies on the αv integrins, adhesion molecules are likely to provide useful targets for antiangiogenic therapy.

Acknowledgments

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