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How Does Extracellular Matrix Control Capillary Morphogenesis?

Minireview

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The function of a tissue depends upon the spatial arrangement of its constituent cells. But control of morphogenesis, the process by which spatial arrangement arises, is poorly understood. One of the simplest model systems used to study tissue development is the formation of capillary tubes by vascular endothelial cells, i.e., angiogenesis in vitro. Cloned endothelial cells retain all of the information necessary to form three-dimensional tubular networks given the correct extracellular cues. At least two such cues, one soluble and the other insoluble, govern this process (reviewed in Ingber and Folkman, 1989a). Soluble molecules that stimulate angiogenesis in vitro include endothelial mitogens such as fibroblast growth factor, as well as growth inhibitors, e.g., transforming growth factor β (Madri et al., 1988). Insoluble factors that promote capillary organization include complex extracellular matrices (ECM; e.g., native collagen gels, plasma clots, Matrigel) and purified ECM molecules such as laminin, fibronectin, and types I, III, IV, and V collagens. ECM and diffusible angiogenic regulators commonly act in concert. However, ECM molecules dominate because they dictate whether individual endothelial cells will proliferate, involute, or differentiate in response to soluble cues.

What molecular mechanisms can explain how ECM molecules control cell responsiveness to soluble factors and switch endothelial cells between growth and differentiation? This question is addressed in a paper in this issue of *Cell* (Grant et al., 1989) and is the focus of our minireview.

Grant and co-workers report two domains in the laminin molecule that mediate endothelial cell alignment and capillary tube formation in the presence of soluble mitogens. One of these domains is the YIGSR sequence in the B1 chain of laminin. As a synthetic peptide, it can rapidly induce a single endothelial cell to organize into a ring-like structure that resembles a capillary lumen in cross-section. This morphological effect is apparently mediated by binding to a specific laminin-binding protein on the cell surface. The other domain is an RGD-containing sequence in the A chain of laminin. This sequence mediates endothelial cell attachment to laminin via binding to a cell surface integrin receptor. Inhibition studies using synthetic peptides and receptor-specific antibodies suggest that the laminin A chain is involved in the initial attachment of endothelial cells whereas the B1 chain mediates cellular reorganization and reorientation within hollow tubes. However, both modes of attachment are necessary for capillary differentiation to occur. Thus, the authors suggest that "distinct sites on laminin working through different receptors act in concert to induce the formation of capillary-like structures in vivo."

This important finding raises a more fundamental question: how does binding of a specific ECM protein domain to an endothelial cell surface receptor convey information that can direct generation of three-dimensional form? Several ECM receptor-mediated chemical signaling pathways similar to those triggered by soluble growth factors have been identified in other cell types. For example, cell spreading on fibronectin activates the phosphatidylinositol cycle in BHK cells (Breuer and Wagener, 1989) and induces intracellular alkalinization in fibroblasts (Schwartz et al., 1989). The platelet integrin, glycoprotein IIb/IIIa, regulates both Na^+/H^+ exchange (Banga et al., 1986) and tyrosine-specific protein phosphorylation (Ferrell and Martin, 1989).

For vascular endothelial cells, little is known about the chemical signaling pathways that are activated by cell binding to ECM. We recently found that fibronectin activates the cell surface Na^+/H^+ antiporter in endothelial cells as it does in fibroblasts, although there currently is no evidence that cytoplasmic pH is involved in control of capillary differentiation. Molecules that stimulate tyrosine phosphorylation also induce angiogenesis in collagen gels (Montesano et al., 1988). But the importance of protein phosphorylation may relate more to early migratory events (when endothelial cells first invade the underlying matrix) than to later stages in which cellular cords reorganize into hollow tubes.

In addition to chemical signaling, ECM molecules may convey regulatory information through binding interactions with cytoskeletal proteins (see Buck and Horwitz, 1987, and references therein). A specific domain within the cytoplasmic portion of the integrin β chain appears to interconnect with microfilaments by binding to the actin-associated molecule, talin. The cell surface laminin-binding protein (which is not an integrin) reportedly binds to actin filaments directly (Brown et al., 1983), although specific binding sequences remain to be identified. In endothelial cells, cell surface laminin-binding proteins colocalize with actin microfilaments (Yannariello-Brown et al., 1988). Interstitial matrix molecules (e.g., collagens) may interconnect with the actin cytoskeleton by binding to integrins or to other cell surface ECM receptors such as heparan sulfate proteoglycans (Rapraeger et al., 1986). Nonerythroid spectrin may participate in the docking of interstitial matrix receptors to cortical microfilaments in endothelial cells (Pratt et al., 1984).

These ECM–cytoskeletal linkages are most likely critical for morphogenesis because hollow tube formation requires remodeling of the cytoskeleton. In addition, cytoskeletal alterations can redistribute regulatory molecules such as mRNA, alter mobility and function of integral membrane proteins, and change nuclear structure (Fey

et al., 1984, and references therein). ECM-dependent changes of cytoskeletal filament assembly and nuclear organization may also alter hormone responsiveness and switch on differentiation-specific genes (Bissell et al., 1982; Nelson et al., 1986).

It is difficult to understand how binding of an ECM molecule to a transmembrane receptor can produce changes of cytoskeletal filament polymerization and orientation. Scattered pieces of the puzzle are just beginning to come together. For example, ECM receptor occupancy appears to yield second messengers that control filament assembly. Phosphoinositides inhibit the actin filament–severing activity of gelsolin (Janmey and Stossel, 1987) and thus actin polymerization may be altered as a result of activation of the phosphatidylinositol cycle by fibronectin. Other diffusible signals (such as cyclic nucleotides, divalent cations, cytoplasmic pH alterations) also modulate cytoskeletal polymerization.

Protein kinases such as those found within focal contact sites (Burridge, 1986) may change cytoskeletal organization by regulating integrin phosphorylation. The cytoplasmic portion of the integrin β chain that mediates binding to talin contains the consensus tyrosine phosphorylation site found in both the epidermal growth factor and insulin receptors (Tamkun et al., 1986). Phosphorylation of this site affects integrin–talin binding as well as integrin–ECM interactions (Buck and Horwitz, 1987). Interestingly, integrin phosphorylation levels change dramatically during differentiation of F-9 teratocarcinoma stem cells (Dahl and Grabel, 1989). Loss of integrin phosphorylation correlates temporally with restructuring of the actin cytoskeleton in this system. However, serine phosphorylation, not tyrosine, appears to mediate this effect.

The difficulty biologists are having in discovering the mechanisms by which ECM molecules transduce pattern-regulating information may lie in our failure to consider the role of resting tension within the cytoskeleton. Tensile forces are generated by contractile microfilaments and resisted by extracellular attachment points; variations in this balance of forces result in alterations of cell and tissue form (Ingber and Folkman, 1988b). Precisely because of their insolubility, ECM molecules resist cell-generated tensile forces and increase isometric tension within the cell when they bind to cell surface receptors. Mechanical stresses can only be transmitted over structural elements that are physically interconnected. Thus, morphogenetic signaling by transmembrane ECM receptors may be based on their ability to experience forces that soluble molecules cannot recognize. Lateral transmission of physical forces through cytoskeletal interconnections in the cell cortex may trigger stretch-activated membrane channels on the endothelial cell surface (Lansman et al., 1987; Olesen et al., 1988). Forces generated within contractile microfilaments and resisted by occupied ECM receptors also may act as feedback to change cytoskeletal filament assembly; polymerization of actin and tubulin is sensitive to tension in cultured cells (Terracio et al., 1988; Dennerll et al., 1988). This model would explain why cell attachment to substrate-adsorbed ECM molecules produces distinct polymerization patterns within the cytoskeleton.

Clear evidence for the role of cell-generated tension in ECM-dependent signaling was recently found by studying angiogenesis in vitro. Simple alterations of a substratum's ability to resist (or not to resist) tensile forces generated by endothelial cells could switch these cells from proliferation to differentiation in the presence of saturating levels of growth factor (Ingber and Folkman, 1989a). Adhesiveness of the substratum was varied by coating it with different densities of purified ECM molecules. High ECM densities resist cell-generated tensile forces and promote extensive cell spreading and growth. In contrast, lower ECM concentrations permit endothelial cell shortening and result in partial retraction of multicellular aggregrates. These cell shape changes turn off cell growth (Folkman and Moscona, 1978) and switch on capillary tube formation. They are not limited to a single type of ECM molecule, which suggests that more than one type of ECM receptor can transmit these mechanochemical cues.

Other studies of in vitro angiogenesis attest to the importance of ECM as a resistor of cell tension and confirm that ECM receptor occupancy per se is not sufficient to convert an endothelial cell monolayer into a network of capillary tubes. Endothelial cells form quiescent tubes when embedded within malleable collagen gels, yet they grow as a monolayer when attached to rigid collagen-coated dishes. Laminin promotes rapid and extensive capillary tube formation when present in a gel (Matrigel), but only induces moderate reorganization when coated on plastic. Furthermore, soluble YIGSR and RGD peptides that clearly occupy ECM receptors produce endothelial cell detachment and are unable to stimulate capillary tube formation. Finally, although substrate adsorbed–YIGSR promotes endothelial cell ring formation, it fails to reorganize cells into tubular networks. Thus, the morphogenetic action of ECM molecules depends on their capacity to both bind specific receptors and resist mechanical loads applied to those receptors. This effect is not limited to capillary differentiation; tension-dependent interactions betwen cells and specific ECM molecules also change tissue form and induce expression of differentiation-specific genes in other cell types, including mesenchyme (Tomasek and Hay, 1984), mammary epithelia (Li et al., 1987), and liver cells (Spray et al., 1987; Ben Ze'ev et al., 1988).

We infer from these findings that ECM insolubility conveys regulatory information to the endothelial cell by resisting tension generated within the intracellular cytoskeleton. If they were completely soluble, ECM molecules would be unable to resist mechanical tension and thus would have no morphogenetic activity. Chemical addresses within ECM molecules clearly mediate this process. However, it is likely that ECM–receptor interactions control capillary morphogenesis based on a mechanism that is mechanochemical rather than purely chemical in nature.

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