

Perspectives series: cell adhesion in vascular biology. Integrin signaling in vascular biology.

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J Clin Invest. 1997;100(1):1-5. <https://doi.org/10.1172/JCI119500>.

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Perspectives Series: Cell Adhesion in Vascular Biology

Integrin Signaling in Vascular Biology

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Previous articles in this series have emphasized the fundamental importance of adhesion receptors in vascular biology. One class of these receptors, the integrins, is necessary for vascular and hematopoietic cell development, angiogenesis, cell migration in response to injury, and extracellular matrix assembly. Furthermore, an integrin specific to platelets and megakaryocytes, $\alpha_{IIb}\beta_3$, is indispensable for hemostasis and has become a validated therapeutic target for antithrombotic drugs. Thus, these widely distributed receptors play prominent roles in normal vascular biology and pathology.

Integrins are noncovalent $\alpha\beta$ heterodimers. Each subunit consists of a relatively large NH_2 -terminal extracellular domain, a single membrane-spanning domain, and a COOH -terminal cytoplasmic tail. So far, at least 17 different integrin α subunits and 8 β subunits have been cloned and over 20 different $\alpha\beta$ pairings have been identified in vertebrate tissues. Integrins were originally identified because of their adhesive properties. Now, multiple lines of evidence indicate that they also function as signaling receptors and that integrin signaling is as vital to vascular cells as is integrin adhesion. The term integrin signaling refers to the capacity of these receptors to transmit information in both directions across the plasma membrane. There has been a recent convergence of scientific interest at this interface of cell adhesion and signaling, and several pertinent reviews are available (1–8). Our purpose here is to outline what is encompassed by the concept of integrin signaling and to present several emerging principles concerning its mechanisms.

What is integrin signaling?

Integrins have been shown to play a role in regulating gene expression and cell growth, differentiation, and survival (2, 9, 10). They accomplish these tasks by a process of outside-in signaling, whereby ligand-occupied and clustered integrins control cell shape and the organization of the cytoskeleton and generate a variety of biochemical signals. Many integrin-triggered reactions, for example activation of protein tyrosine kinases, such as pp60^{Src} , $\text{pp125}^{\text{FAK}}$, and pp72^{Syk} , and activation of

phosphatidylinositol 3-kinase and MAP kinases, are shared with those generated by more traditional agonist receptors, such as those for growth factors and cytokines. Thus, integrins are bona fide signaling receptors with the added twist that their ligands also mediate cell anchorage. As a result, integrin signaling is initially focused at topographically localized regions of the plasma membrane where cell–cell and cell–extracellular matrix contact take place. This serves to specify anchorage-dependent changes in cell shape, polarization, and motility (11).

One other contrast with traditional agonist receptors is that integrins initiate an extracellular effector response (e.g., cell anchorage) coincident with ligand engagement. For most integrins, ligand binding is tightly regulated by cellular signaling mechanisms through a process referred to as integrin activation or inside-out signaling. This translates intracellular signals into extracellular work. Since integrin ligands are either multivalent extracellular matrix proteins or transmembrane counterreceptors, integrin activation can reflect an increase in the true affinity of the receptor for ligand or an increase in avidity. Of course, changes in affinity and avidity are not mutually exclusive, and the relative contributions of each to integrin activation probably vary with the integrin and the cell type. For example, affinity modulation seems largely responsible for the initial binding of soluble fibrinogen or von Willebrand factor to $\alpha_{IIb}\beta_3$ during platelet aggregation (12). In contrast, avidity modulation is a major factor in the activation of $\alpha_L\beta_2$ in leukocytes, leading to the interaction of $\alpha_L\beta_2$ with ICAM-1 on endothelial cells and *trans*-endothelial leukocyte migration (13, 14).

A modification of true affinity implies a structural change intrinsic to the integrin heterodimer that results in a greater strength of ligand binding. Different affinity states have been characterized in integrins of the β_1 , β_2 , β_3 , and β_7 classes, principally through the use of cell activation-specific soluble ligands (12, 15–17). These different states probably reflect conformational changes within and between the receptor subunits that affect the shape or accessibility of the ligand-binding interface. The details of these changes should soon come into sharper focus because of the advent of genetic strategies to identify integrin activators and suppressors (18–20), and preparative and analytical techniques with which to model and solve integrin structures (21–25).

Affinity modulation as a form of signal transduction is directly relevant to vascular biology. First, several antithrombotic drugs (e.g., aspirin, ticlopidine, clopidogrel, phosphodiesterase inhibitors) work by regulating the capacity of platelet signaling mechanisms to initiate conformational changes in $\alpha_{IIb}\beta_3$ (26). Second, there may be situations in which an integrin in a high-affinity state is too much of a good thing. A re-

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Received for publication 12 May 1997.

J. Clin. Invest.

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0021-9738/97/07/0001/05 \$2.00

Volume 100, Number 1, July 1997, 1–5

cent quantitative analysis of the relationship among fibroblast migration, integrin affinity, and substrate density showed that when integrin affinity is high, blockade of migration occurs when substrate and integrin densities are also high (27). Conceivably, therefore, inappropriate activation of integrins might actually inhibit leukocyte, endothelial cell, or smooth muscle cell migration. This may be one explanation for the existence of suppressor pathways that oppose integrin activation (20). Finally, the capacity of cells to assemble a fibronectin matrix is regulated by the activation state of integrins, a finding of potential importance during vascular responses to injury (28).

Avidity modulation implies a change in functional affinity whereby the interaction between receptor and ligand are influenced by rebinding or chelate effects. The avidity of integrins is likely promoted by their clustering or multimerization within the plane of the plasma membrane. Various light microscopic techniques have detected clusters of ligand-occupied integrins in adherent cells, including platelets, endothelial cells, and vascular smooth muscle cells. These clusters can take the form of smaller focal complexes, which assemble during filopodial and lamellipodial extension (29–31), or larger focal adhesions, which are connected to actin stress fibers and assemble during the later stages of cell spreading (32). Filopodia, lamellipodia, and focal adhesions are regulated by members of the Rho family of GTPases (33), exemplifying one facet of a likely complex relationship between Rho signaling and integrin function. Focal complexes and focal adhesions are dynamic structures which contain a panoply of signaling molecules and cytoskeletal proteins, and their coordinated assembly and disassembly are presumably essential for vascular cell migration (4, 11, 32).

Emerging principles of integrin signaling

How do integrins interact with the signaling machinery of cells? Current models envision a hierarchical organization to

integrin signaling, which necessitates that some signaling molecules interact closely, if not directly with integrin subunits. Several general principles are beginning to emerge regarding this proximal aspect of integrin signaling.

Integrin cytoplasmic domains play a pivotal role in integrin signaling. This is a conceptually appealing idea, since the cytoplasmic tails of integrin α and β subunits are directly accessible to the intracellular signaling apparatus. Except for the β_4 cytoplasmic tail, which contains > 1,000 amino acid residues, integrin tails range in size from ~ 20 to 70 residues. Mutations or truncations of specific membrane-distal sequences in β cytoplasmic tails can disrupt integrin-triggered signaling and cytoskeletal organization (34–39). Furthermore, overexpression of isolated β cytoplasmic tail chimeras can profoundly suppress both inside-out and outside-in signaling, possibly by titration of critical regulatory molecules (40–42). Conversely, when clustered or highly overexpressed, these β tail chimeras can themselves generate some of the biochemical signals, such as tyrosine phosphorylation of FAK, usually triggered by integrin ligation (40, 42). The roles of the α cytoplasmic tails seem even more complex. For example, deletion of certain membrane-distal α tail sequences can result in constitutive biochemical signaling (38) and at the same time lead to reduced cell adhesion (43). Thus, α tails exert both positive and negative influences on integrin signaling.

The membrane-proximal portions of integrin cytoplasmic domains are highly conserved. Truncations that disrupt the most membrane-proximal five to seven residues of either the α_{11b} or β_3 cytoplasmic tail markedly increase ligand binding affinity, most likely due to disruption in intersubunit interactions that normally maintain a default low-affinity state (44, 45). Indeed, selected point mutations in this region induce ligand binding and initiate spontaneous tyrosine phosphorylation of FAK (46). Accordingly, the membrane-proximal integrin hinge

Table I. Integrin Tail-binding Proteins

Protein	Integrin tail partner	Notable features	Reference
Calreticulin	α^*	Expression correlates with integrin-mediated cell adhesion; present in many subcellular locations	50–53
F-Actin	α_2 only	Structural cytoskeletal protein	66
Calcium- and integrin-binding protein (CIB)	α_{11b} only	Sequence homology to calcineurin B; contains two EF-hand motifs	67
Talin	α_{11b} ; β	Structural cytoskeletal protein	68, 69
α -Actinin	β	Structural cytoskeletal protein	70
Skelemin	β	A myosin and intermediate filament-associated protein	71
pp125 ^{FAK} (focal adhesion kinase)	β	Protein tyrosine kinase localized to focal adhesions	72
p59 ^{LK} (integrin-linked kinase)	β	Contains ankyrin repeats and serine threonine kinase domain; overexpression inhibits cell adhesion and induces anchorage-independent growth	73
Paxillin	β_1	Adapter with SH2 and SH3 binding motifs and LIM domains	74
ICAP-1	β_1 only	Cell adhesion via β_1 modulates phosphorylation state of ICAP-1	D. Chang, personal communication
Filamin	β_2	Structural cytoskeletal protein	75
Cytohesin-1	β_2 only	Contains Sec7 and PH domains; guanine nucleotide exchange activity for ADP-ribosylation factor; overexpression increases $\alpha_1\beta_2$ -mediated adhesion	48, 76
β_3 -Endonexin	β_3 only	Overexpression increases $\alpha_{11b}\beta_3$ affinity and adhesive function	49, 77

*Unless specified otherwise, the integrin-binding protein has been shown to bind to more than one type of α or β subunit.

region regulates bidirectional integrin signaling.

Recently, a plethora of proteins has been identified that can bind directly to integrin cytoplasmic tails, at least in vitro. Some of these proteins can bind to integrin α tails and others to β tails. Some can bind to more than one type of α or β subunit, others to only a single type (Table I). In the case of one of these, β_3 -endonexin, selective binding to the β_3 tail, but not to the β_1 or β_2 tail, is due to an NITY motif that is specific for the β_3 tail (47). In most cases, identification of these binding proteins has outstripped the characterization of their biological functions, and further analyses of their roles in vivo are required. Nonetheless, the list in Table I suggests intriguing and complex relationships between integrin tail-binding proteins and integrin signaling. For example, some of these proteins are cytoskeletal structural proteins (α -actinin, filamin, talin), while others possess kinase activity (pp125^{FAK}, ILK), guanine nucleotide exchange activity (cytohesin-1), or function as adapters (e.g., paxillin). Interestingly, overexpression of two different tail-selective binding proteins, cytohesin-1 and β_3 -endonexin, results in specific activation of the adhesive function of $\alpha_1\beta_2$ and $\alpha_{IIb}\beta_3$, respectively (48, 49). Finally, calreticulin can bind to the membrane-proximal portion of α cytoplasmic tails (50). Although this protein can be found in more than one subcellular location and may have several functions even with respect to adhesion receptors and cytoskeletal proteins (51, 52), it is noteworthy that calreticulin-null embryonic stem cells are deficient in integrin-mediated cell adhesion and calcium influx (53).

Integrin signaling may also involve the association of integrins with other transmembrane proteins. β_3 integrins appear to specifically associate with CD47 (integrin-associated protein). This association may be involved in the regulation of neutrophil phagocytosis in response to certain ligands (54), and mice lacking CD47 show defects in neutrophil function (55).

The tetraspanin class of transmembrane proteins, such as CD9, CD81, and CD63 can be coprecipitated with certain integrins from detergent extracts of cells (56–58). Since some of these associations can be induced by antibody cross-linking of integrins, they may regulate some aspects of integrin signaling and cell migration. Recently, the physical association of phosphatidylinositol 4-kinase with tetraspanins and integrins has been reported, providing a possible connection between integrins and phosphatidylinositide metabolism (59).

Some β_1 integrins seem to promote cell growth whereas others promote cell differentiation. This has been shown to correlate with the capacity of these integrins to activate the MAP kinase pathway via the adapter, Shc (60). Furthermore, the growth-enhancing integrins form physical complexes with Shc. This interaction does not involve the integrin cytoplasmic tails but rather correlates with an association between the extracellular or transmembrane domain of the integrin α subunit and caveolin, a protein that may scaffold a variety of signaling proteins (61). Thus, some interactions of integrins with other proteins may be independent of the cytoplasmic domains and may play critical roles in integrin signaling.

The anchorage dependence of vascular cell growth, differentiation, and survival suggests that integrins play unique and indispensable roles. One possibility is that integrins play a permissive role in growth factor signaling pathways. In this view, integrin ligation may be required for normal growth factor regulation of cell growth and survival. Integrins appear to collaborate with growth factors in many ways (8, 62). First, they may regulate the availability of substrates for enzymes activated by

growth factors (63). Second, growth factor receptors often partition into complexes assembled by integrins, and in these complexes they may become activated and signal more efficiently (62, 64). Third, cell adhesion is required for critical downstream events in growth factor signaling, e.g., activation of the MAP kinase pathway and traverse through the cell cycle (5, 9). Finally, integrins control cell shape, which in itself is an important determinant of cell growth and differentiation (10, 65).

Certain aspects of integrin signaling are integrin- and cell type-specific. This point seems self-evident but it is worth emphasizing. The combinatorial repertoire of extracellular ligands, integrins, and intracellular signaling molecules differs from one cell to another and even within a given cell at various times. While it contributes to the diversity and specificity of the adhesion and signaling responses of integrins, it also complicates the task of teasing out unifying principles. Accordingly, caution is warranted in extrapolating results from one cell type to another and from experiments with integrin inhibitors in the test tube or in animals to clinical trials in humans.

In conclusion, both the adhesive and signaling functions of integrins are critical for their biological activities. Within the vasculature, integrin signaling events play central roles in angiogenesis, cell migration during development and wound repair, inflammatory responses, and hemostasis. In addition, integrins may contribute to the pathogenesis of vascular disease by promoting these same functions at the wrong time or in the wrong place. Examples include platelet thrombus formation after rupture of an atheromatous plaque, vascular smooth muscle cell migration during restenosis after coronary angioplasty, and angiogenesis in diabetic retinopathy. The direct inhibition of ligand binding to integrins is a therapeutic strategy that is already being reduced to practice. Once the details of integrin signaling are established, they may provide additional molecular targets for drug development and therapeutic intervention.

Acknowledgments

Cited studies from the authors' laboratories were supported by grants from the National Institutes of Health (HL-56595, HL-57900, HL-48728, HL-31950, AR-27214) and from Cor Therapeutics, Inc.

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