INSIDE-OUT SIGNALING IN INTEGRINS

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Abstract

Rapid and precisely controlled changes in cell adhesion are a hallmark of many basic physiological processes. These include: (1) leucocyte migration, which occurs during infiltration into inflammatory sites, and (2) platelet aggregation. Integrins are a family of adhesion molecules whose function is controlled by the cells that express them. This property makes them particularly suitable for those situations that demand flexibility of cellular adhesive function. Regulatory signals that originate within the cell cytoplasm are transmitted to the external ligand-binding domain of the receptor to control integrin function. The generation and transmission of these "inside-out" signals are subjects of recent interest that will be summarized in this review.

Introduction

Integrins are a widely distributed family of cell surface molecules that mediate cell adhesion (Table 1).[1-4] They interact with many extracellular ligands and so are involved in a range of physiological processes, including embryogenesis, morphogenesis, wound repair, inflammation, tumour cell migration and leucocyte trafficking. Their ligands include extracellular matrix proteins (e.g., fibronectin, collagen, laminin), other cell surface receptors (e.g., members of the immunoglobulin superfamily, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1), and soluble proteins (e.g., fibrinogen, inactivated complement component C3 (iC3b)) (Table 1).

Table 1. The integrin family.

Integrin	Alternative Names(s)	Ligand(s)	Distribution
a1b1	VLA-1, CD49a/CD29	collagen, laminin	widespread
a2b1	VLA-2, CD49b/CD29	collagen, laminin	widespread
a3b1	VLA-3, CD49c/CD29	laminin-5, fibronectin (?),collagen (?)	widespread
a4b1	VLA-4, CD49d/CD29	fibronectin, VCAM-1	lymphocytes, muscle, monocytes/ macrophages, neural crest cells, fibroblasts
a5b1	VLA-5, CD49e/CD29	fibronectin	widespread
a6b1	VLA-6, CD49f/CD29	laminin, fertilin	widespread
a7b1	VLA-7	laminin	?
a8b1	VLA-8	fibronectin, vitronectin, tenascin	neural

a9b1	VLA-9	tenascin	?
avb1	fibronectin, vitronectin	epithelial cells	
aLb2	LFA-1, CD11a/CD18	ICAM-1, ICAM-2, ICAM-3	leucocytes
aMb2	Mac-1, CD11b/CD18	fibrinogen, iC3b, ICAM-1, factor X	monocytes, granulocytes, NK cells, cytotoxic T lymphocytes
aXb2	p150,95,CD11c/CD18	fibrinogen, iC3b	monocytes, granulocytes, activated B lymphocytes
aIIbb3	gpIIb/IIIa	fibrinogen, fibronectin, vWF, vitronectin	platelets, megakaryocytes
avb3	VnR	fibrinogen, fibronectin, vWF, vitronectin,thrombospondin, osteopontin, collagen	endothelium, tumour cells
a6b4		laminin-1, laminin-5	epithelial cells
avb5		vitronectin, fibronectin (?)	carcinoma cells
avb6		fibronectin	?
a4b7		VCAM-1, , fibronectin	MadCAM-1 activated B and T lymphocytes, monocytes/macrophages
aEb7		E-cadherin	intraepithelial lymphocytes
avb8		vitronectin	?

Abbreviations: VCAM-1 = vasculatr cell adhesion molecule-1; ICAM = intercellular adhesion molecule; iC3b = inactivated complement component C3; vWF = von Willebrand factor; MadCAM-1 = mucosal addressin cell-adhesion molecule

Members of the integrin family are heterodimers, composed of a larger a chain (120 to 180 kD) and a smaller b chain (90 to 110 kD), each of which crosses the cell membrane once. Both chains have amino-terminal extracellular domains, which fold and intertwine to form a binding “pocket” that accommodates their specific ligand(s). Each chain then has a hydrophobic transmembrane region that connects to an intracellular cytoplasmic tail, which, with the exception of the b4 chain, is small and conserved through evolution. The cytoplasmic tails interact with the cytoskeleton through a series of linking proteins that include talin and vinculin.[5,6] Also co-distributed at this site of receptor-cytoskeletal interaction is a collection of signaling proteins including many kinases and GTPases. The activities of many of these signaling molecules are altered

following the integrin-ligand interaction and the clustering of the receptors at the cell surface.[7-9]

Integrins act as transmembrane linkers between intracellular and extracellular proteins and transmit signals across the cell membrane. In fact, while integrins are usually classified as "adhesion molecules," they are also important "signaling molecules."[2] Signal transduction through integrins occurs in two directions - moving from the extracellular microenvironment into the cell cytoplasm ("outside-in signaling") and from the cytoplasm out to the extracellular domain of the receptor ("inside-out signaling").[2] This brief review summarises some recent observations that have yielded insights into the mechanisms underlying inside-out signaling through integrins. Outside-in signaling through integrins has been the subject of several recent reviews.[7-9]

Integrin Function is Regulated

One striking property of integrins is called "affinity modulation."[10] This refers to the fact that the strength with which they bind to their ligand(s) can be rapidly altered. Resting integrins have low affinity for their ligands ("inactive"), but appropriate stimuli can convert them to high affinity ("active") receptors. This enables cells to quickly adjust their adhesive phenotype without changing the type or number of adhesion molecules that they express. This ability makes integrins crucial participants in a variety of important events, some of which will be discussed in the next section. Control of this dynamic process resides within the cell itself, but the necessary adjustments must then be transmitted to the ligand-binding domain of the receptor. This transfer of regulatory information constitutes the "inside-out' signals.

When is Dynamic Regulation of Integrins Useful?

Cell migration is an example of a physiological event that is dependent on rapid, controlled alterations in integrin-mediated cell adhesion. It involves repeated cycles of attachment (at the leading edge of the cell) and detachment (at the trailing edge) as the cell moves in a directed fashion, and this cyclical process is reliant on coordinated activation/deactivation of integrin function at those sites. Many basic processes utilise cell migration, and these include wound healing, embryogenesis, tumour metastasis and inflammation. A well-studied example is that of leucocyte extravasation at sites of inflammation in response to a source of pro-inflammatory cytokines and chemoattractants. The adhesive events involved in this form of cell migration have been elucidated in the last few years. At the point where extravasation is initiated, the resting integrins on circulating, non-adherent leucocytes are activated, which in turn stabilises the preliminary attachments that occur between the leucocytes and the adjacent endothelial cells. The activated integrins then further participate in the subsequent movement of leucocytes into the extravascular tissue. A number of other adhesion molecules (including selectins and immunoglobulin superfamily members) are also involved in this process, and this complex series of events has been summarized in recent reviews.[11,12]

Platelet aggregation is an example of a different type of fundamental process that is also dependent on integrin affinity modulation. Aggregation of platelets relies on their

cross-linking by fibrinogen, a soluble plasma protein which binds to the integrin αIIbβ3, the most abundant platelet cell surface protein. Resting platelets do not aggregate because their aIIbb3 is in the inactive, low-affinity conformation which fails to bind measurable amounts of soluble fibrinogen. Platelet activation by specific agonists (e.g., thrombin, ADP, collagen) activates αIIbβ3, leading to high-affinity binding of soluble fibrinogen, and cross-linking and aggregation ensues.[13-16]

The assembly of a fibronectin matrix around certain cells is a further example of the relevance of integrin affinity modulation. Fibroblasts and other adherent cells are enveloped by a complex sheet of interlinked fibronectin fibrils during growth *in vitro*. This process only occurs with living cells and is thought to represent the *in vitro* equivalent of the laying down of insoluble fibronectin in the extracellular matrix *in vivo*.[17-18] The fibronectin provides crucial positional information for cell migration during embryogenesis, leucocyte extravasation and wound healing. Formation of a fibronectin matrix also has implications for cancer, as it has been known for some time that cells transformed *in vitro* with oncogenic viruses lose their ability to form and retain their fibronectin matrix.[19-20] In most cases, matrix assembly is dependent on the expression of functional integrin α5β1 (the "classical" fibronectin receptor). Inhibiting the binding of fibronectin to α5β1 blocks matrix assembly, and overexpression of α5β1 in cells induces increased fibril formation.[21] Conversely, certain cells express abundant α5β1 but appear unable to assemble a fibronectin matrix. In addition, other receptors seem capable of supporting matrix assembly, based on studies of α5β1-"knockout" mice.[22] This is not all that surprising when you consider the number of other potential fibronectin receptors in the integrin family (Table 1). The resolution of these apparent paradoxes now appears closer with the recognition of two distinct requirements for effective matrix assembly. The first is expression of a fibronectin receptor (usually but not necessarily α5β1) that is in the activated conformation so that it can bind soluble fibronectin with high affinity. The second is the involvement of events that occur following occupancy of the activated receptor by fibronectin. These events are not yet well defined, but they involve the cytoplasmic tail of the integrin b chain and require an intact actin cytoskeleton.[23]

How Are the Activating, Inside-Out Signals Generated?

Much of what is known about integrin activation comes from studies of the platelet receptor αIIbβ3 (GPIIb-IIIa). The reasons for this concentration of knowledge include the ease of isolating platelets, the ease of purifying the receptor, the existence of well-defined platelet activators (e.g., thrombin, adenine nucleotides, phorbol esters), and the ability to directly measure changes in receptor affinity for ligand using soluble fibrinogen (or fibronectin) binding assays.[15,16] Patients with Glanzmann's thrombasthenia have a bleeding tendency due to defective clot formation secondary to impaired platelet aggregation. In most cases they have absent or little expression of αIIbβ 3 on their platelets. However, a minority of patients express normal or near-normal levels of αIIbβ3 that is unable to bind fibrinogen (or other ligands) with high affinity.(24-29) The DNA encoding these receptors contains point mutations in certain regions of the

molecule, which in turn has provided important insights into the acquisition of high-affinity ligand binding (see below).

A potent platelet agonist is thrombin. The platelet and αIIbβ3 activation that follows thrombin binding to its specific receptor occur through G proteins, which in turn activates phospholipase C, inducing phosphoinositide hydrolysis and the production of inositol 1,4,5-triphosphate and diacylglycerol.[30-32] Concurrently, there are changes in cytosolic calcium and activation of cellular protein kinases (including protein kinase C). Direct activators of protein kinase C (such as phorbol esters) induce phosphorylation of the b3 (but not αIIβ) cytoplasmic tail. However, the stoichiometry of the phosphorylation appears to be too low to account for the receptor activation.[33] The cytoplasmic tails of β 2 integrins are also phosphorylated following receptor activation by certain agonists, but the activation is not affected by mutation of the relevant phosphorylation site.[34] The activation of αIIbβ3 by these agonists is platelet-specific, as it cannot be reproduced in HEL cells (which express both αIIbβ3 and a functional thrombin receptor[35]) or Chinese hamster ovary (CHO) αIIbβ3-transfectants.[36]

Occupancy and/or ligation of other cell surface molecules also have positive or negative regulatory effects on integrin function. Ligation of avb3 with specific antibodies inhibits α5β1-mediated phagocytosis of fibronectin-coated particles by the same cells (this process requires high-affinity α5β1 receptors). The signals generated are dependent upon serine-threonine kinases as well as the presence of the β3 chain cytoplasmic tail.(37, 38) Ligation of platelet/endothelial cell adhesion molecule 1 (PECAM-1) also induces the function of β1 and β2 integrins expressed by the same cells, although the precise nature of the activating signal is not clear.[39, 40]

In contrast to these acute changes in receptor function, some cells maintain a repertoire of activated integrins on their surface. For example, the α5β1 expressed by adherent cells (such as CHO cells) is intrinsically activated, and the maintenance of this active state is dependent on intracellular signaling.[41] Certain other cells normally express inactive α5β1.[42] Induction of differentiation in monocytic cells is also associated with activation of α5β1, in parallel to the adoption of an adherent, macrophage phenotype.[43] This may be a key event in the acquisition of phagocytic function during monocyte differentiation.

The cascades of activating signals that are initiated in turn act upon the cytoplasmic tails of the integrins in order to produce their net effect. Several models have been proposed to explain precisely how the exchange of information occurs.[10, 44] One model proposes that the signals induce alterations in the cytoplasmic tails themselves (e.g., phosphorylation (see above) or another post-translational modification, or alterations in the interactions between the α and β tails). It is noteworthy that thrombin aggregation of platelets induces cleavage of the cytoplasmic domain of the β3 chain by calpain, which is an intracellular calcium-dependent protease.[45] Four of the cleavage sites flank NXXY motifs that are known to be important in integrin inside-out signaling,[46] which suggests that this is a potential means of regulating integrin activation. Another model proposes the existence of a intracellular “repressor” protein, which is normally bound to the cytoplasmic tail(s) but is removed during receptor activation. A third model proposes an “activator” protein that associates with the integrin during activation. Limiting quantities of these modifying factors appear to reside within

the cytoplasm of cells.[47] A recently isolated novel intracellular protein, called β3-endonexin, is a candidate for either of these roles. It binds specifically to the integrin β3 tail,[48] and a point mutation in the β3 tail (S752P) that inhibits this binding has also been found in patients with a variant of Glanzmann's thrombasthenia characterised by defective activation of aIIbb3.[49]

Membrane proximal residues in the β3 cytoplasmic tail (Leu717 - Asp723) are also involved in receptor activation, since their deletion produces a constitutively activated receptor.[50] These residues are highly conserved amongst integrins. The aIIb cytoplasmic tail is also involved in receptor activation because its deletion also induces high affinity ligand binding.[51] This property also appears to reside within the membrane proximal portion of the tail, and has been mapped to the GFFKR motif that is highly conserved across the integrin family.[41]

How Are the Activating Signals Transmitted to the Extracellular Domain of the Integrin?

Electron microscopy studies suggest that an integrin consists of a globular "head," formed by the amino terminal regions of the α and β chains, attached to the cell by "legs" formed by the carboxyl ends of each chain.[52] The complex tertiary structure of each of the chains is stabilized by disulfide bonds that bridge between a number of highly conserved cysteine residues.[53,54] Further electron microscopy studies suggest that ligand binding occurs in this "head" region, at a site that is most distant from the cell membrane (and the cytoplasmic tails).[55] Hence, activating structural alterations within or adjacent to the cytoplasmic tails need to be transmitted the full length of the molecule in order to mediate the change in ligand accessibility.

One mechanism to account for this transmission of signals is via long-range propagated conformational changes that ultimately activate receptor function by exposing the ligand-binding region.[56] Measurable changes in the biophysical properties of αIIbβ3 occur during platelet activation,[57] and a number of monoclonal antibodies have been produced that recognize conformation-sensitive epitopes in the same receptor.[58-62] Some of these antibodies possess the dual properties of binding with higher affinity to the occupied form of the receptor as well as themselves inducing receptor activation. The epitope of one such "activating" antibody has been mapped to the membrane proximal region of the extracellular domain of the β3 chain.[56] This suggests that the activating conformational change induced by the binding of this antibody is transmitted along the length of the molecule to the distal ligand-binding site. A similar or identical process may occur during the propagation of "inside-out" signals. In addition, the conformational change detected by such an antibody following receptor occupancy may be analogous to that which mediates the aforementioned "outside-in" signals. Similar "activating" antibodies directed against other integrins, including members of the β1 and β2 subfamilies, have also been described.[63-69]

"Inside-Out" Signals Produce Changes in the Conformation of the Integrin Ligand-Binding Pocket

The outcome of this generation and propagation of "inside-out" signals is high-affinity binding of the integrins' ligands. Conversion to high-affinity binding seems to be an intrinsic property of the receptor itself, rather than secondary to removal of extrinsic factors that were blocking access to the receptor (such as other receptors and/or soluble factors).[36] In most if not all cases the change in the external domain of the receptor appears to be conformational, and it can occur very rapidly. The result is better access of macromolecular ligands to the binding "pocket" that is formed by the intertwined α and β chains of the receptor.[70] The site of this binding pocket has been mapped in some detail, and it contains some key residues that are mutated in certain variant forms of Glanzmann's thrombasthenia.[71] Small peptides that mimic the binding sites on ligands (e.g., RGD-containing peptides) can readily gain access to the ligand-binding region.[58] In some cases the "inactive" form of an integrin can also bind ligand with measurable, albeit low, affinity (e.g., a5b1 and soluble fibronectin),[42] and this low-affinity binding can be utilized for cell adhesion in certain settings.[43]

Conclusion

Cells can regulate their adhesive phenotype by altering the function of the integrin adhesion receptors that they express. This ability is central to the control of important and varied physiological and pathophysiological processes. The basis of this regulation is intracellular signals that are transmitted to the extracellular domain of the receptor and induce changes in receptor conformation. The initiation, coordination and transmission of these "inside-out" signals have all been studied in the last few years, and significant progress has been made in their understanding at the molecular level.

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